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CHEMICAL MODIFICATION OF VISCOSE FIBRES BY ADSORPTION OF CARBOXYMETHYL CELLULOSE AND CLICK CHEMISTRY

Master's thesis for the degree of Master of Science in Technology submitted for inspection, Espoo, 11 August, 2014.

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Abstract

Functionalization of cellulosic materials to achieve new and advanced properties is a widely explored research area. This thesis is focused on the novel approach for modification of cellulosic materials by the combination of adsorption of carboxymethyl cellulose (CMC) onto cellulose surface and the copper-catalyzed azide-alkyne cycloaddition (CuAAC) “click” reaction.

The literature part gives an overview on the basics of cellulose chemistry, chemical functionalization of cellulose, as well as explains the principle of the chemical conjugation of carboxylic groups obtained by formation of amide bonds. The chapter continues with the phenomenon of the polyelectrolyte adsorption onto cellulose, takes a look at the viscose production process and, finally, it introduces the novel field of “click” chemistry.

In the experimental part, methods and techniques applied are described. The experimental part focuses on the evaluation of the adsorption efficiency of CMC and its derivatives onto viscose in different reaction conditions, as well as on examination of chemical and mechanical stabilities of the modification. CMC functionalization was analyzed with spectroscopic techniques and elemental analysis. The effect of various reaction conditions on adsorption was studied and quantified by means of the phenol-sulphuric acid method for the estimation of total adsorbed carbohydrates. Chemical and mechanical stabilities of the modified CMC and fibres were studied by the alkaline and wet frictional treatments, respectively.

The main findings revealed that chemically modified viscose fibres (cellulose II) behave differently from cellulose wood pulp and cotton (cellulose I) in respect of polysaccharide adsorption. The functionalization of CMC resulted in clear changes in the molecular structure of CMC, appearing as an increase in nitrogen content. In addition, spectroscopic analyses revealed characteristic signals of alkyne, azide and amide bonds indicative of successful grafting reactions. Adsorption of CMC onto viscose fibres was studied with Raman spectroscopy and x-ray photoelectron spectroscopy (XPS), and revealed changes on the surface of the viscose. The click reaction was demonstrated via fluorescent labelling. Moreover, the study on chemical and mechanical stabilities showed reasonable results. In general, combination of polysaccharide adsorption and click chemistry can be considered as a promising method for introducing new functionalities to the viscose fibres.

Keywords regenerated cellulose, viscose, carboxymethyl cellulose, CMC, adsorption, chemical modification, click chemistry, CuAAC, copper catalyst

FOREWORD

This thesis was conducted within the Biorefineries Research Group at Department of Forest Products Technology in cooperation with Kelheim Fibres GmbH. I am grateful to all the group members for creating an incredibly pleasant working environment and their friendly and helpful attitude.

I would like to express my gratitude to my supervisor Professor Herbert Sixta for the opportunity of joining the team and working on a topic of big interest to the industrial manufacturer. I would also like to thank my instructor Dr. Ilari Filpponen for his valuable advice. Their guidance and motivating comments are highly appreciated. My gratitude also goes to M.Sc. Jiaqi Guo as well as the experts Mirja Reinikainen, Leena-Sisko Johansson and Joseph Campbell for their help with analysis techniques. Furthermore, the Microanalytical Laboratory of University of Vienna and Laboratory of Organic Chemistry of University of Helsinki are thanked for the service provided. Finally, the R&D department of Kelheim Fibres GmbH is acknowledged for the cooperation and financial support.

Last but not least, I am most thankful to my dearest family and friends for their support, encouragement and care that are of immense value to me.

Espoo, 11 August, 2014

Olga Anufrijeva

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1 INTRODUCTION

The use of cellulose fibres in innovative areas of materials science has recently gained considerable attention due to potential advantages they possess: their biorenewable character, recyclability and availability in the variety of forms as well as low cost (Qiu, Hu 2013). Functionalization of polymer materials to achieve intelligent properties has been a popular research topic in the field of cellulose chemistry as well as has become of greater interest in textile chemistry. Among many existing chemical methods for the modification of cellulose-based materials, including periodate oxidation (Siller et al. 2013) and incorporation of nanoscale particles (El-Gabry, Allam & Hakeim 2013), one that has become an attractive and high-potential alternative is cellulose functionalization by the combination of polyelectrolyte adsorption onto cellulose surface and the "click" chemistry reactions. The latter novel field of chemistry follows the principles of green chemistry, requires mild reaction conditions and causes no reducing effect on physical properties of materials.

Irreversible adsorption of various polysaccharides, including water-soluble hemicelluloses and cellulose derivatives onto cellulose surface has been considered a non-destructive method for the introduction of new conjugation sites on cellulosic materials. The adsorption of cellulose-like ionic polysaccharides, including carboxymethyl cellulose (CMC), on cellulose kraft pulps, nanofibrillar cellulose (NFC) and textile fibres has been demonstrated in the literature (Laine et al. 2000; Fras Zemljič et al. 2006; Filpponen et al. 2012; Junka et al. 2014; Hensley, Inks 1959), where anionically charged CMC has been found to adsorb on cellulose. The adsorption of different compounds onto cellulose fibres may increase with the increased content of acidic groups, but there is little research regarding this in the field of textile chemistry, and especially with viscose being the material in question.

This thesis is focused on the novel approach for modification of cellulosic materials, in which the first step implies the activation of cellulose surface that is obtained by the physical binding of the carboxymethyl cellulose chains with “clickable” functional groups on the cellulose surface. Next step is the surface modification achieved by the click reaction, where the desired molecule is covalently attached to the modified CMC that is already adsorbed on the surface. The method allows introducing practically any azide- or alkyne-containing molecule, the desired property carrier, on the surface of a cellulosic material (Filpponen et al. 2012). The adsorption behaviour is not yet entirely understood, thus the main tasks investigated within this work are the adsorption efficiency of CMC onto viscose fibres and the stability of performed click chemistry modifications.

2 LITERATURE OVERVIEW

2.1 Cellulose chemistry

Cellulose, the structural component of plant cells, is the most abundant and important natural substance produced by living organisms. It is distributed in all plants from higher ones to primitive organisms, such as sea weeds, fungi and bacteria. Cellulose is one of the most typical organic polymers with around $1.5 \cdot 10^{12}$ tons of annual production which serves as the basis for many technical products (e.g., paper, films, fibers, additives) and is attained from wood by pulping processes on industrial scale (Fengel, Wegener 2011a; Klemm et al. 2005).

Cellulose is a linear polymer composed of two repeating anhydroglucose units (AGU) which are covalently bound by β-(1,4)-glycosidic linkages. Every second AGU ring is rotated 180° in the plane, thus accommodating the preferred bond angles of the acetal oxygen bridges. In this manner, two adjacent glucose units, linked by elimination of one molecule of water between their hydroxyl groups at carbon 1 and carbon 4, define the disaccharide cellobiose, as illustrated in Figure 2-1. The chain length of cellulose molecule that is expressed as degree of polymerization (DP), a number of monomeric units in a polymer, varies from 250 to 15300 repeating units depending on the origin and treatment of the raw material. The DP-value is strongly affected by intensive chemical treatment methods, such as pulping and bleaching, along with delignification or even atmospheric oxygen, resulting in reduction in cellulose chain length (Klemm et al. 2005; Fengel, Wegener 2011a).

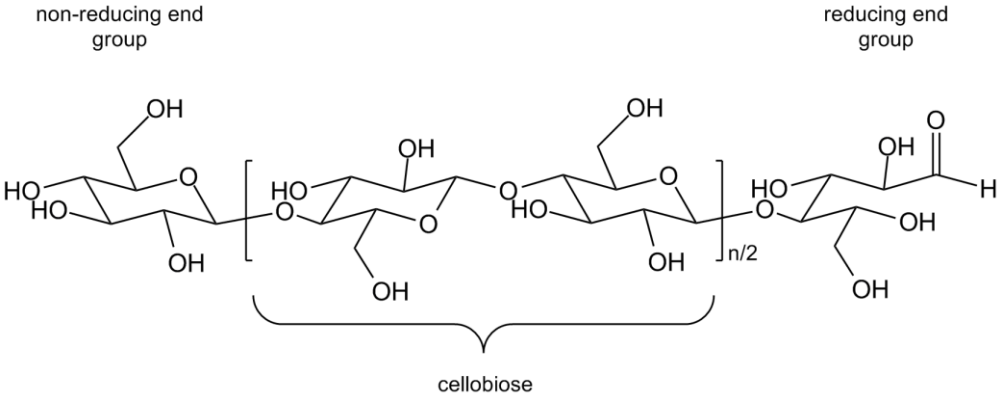


Figure 2-1. Molecular structure of cellulose

The reactions and properties of cellulose are determined by several aspects: inter- and intramolecular interactions, cross-linking reactions, chain lengths as well as the distribution of functional groups on the repeating units along the polymer chains. Although OH-groups are present at both ends of the cellulose chain, they behave differently due to the chemical nature. The OH-group at the C1-end is an aldehyde hydrate group with reducing properties deriving from the ring formation by an intramolecular hemiacetal linkage. The C4-OH group, on the other hand, is an alcoholic hydroxyl and, therefore, non-reducing. By virtue of specific molecular structure, cellulose is characterized as a hydrophilic, chiral and degradable biopolymer with broad chemical variability deriving from the high donor reactivity of OH-groups that determine extensive hydrogen bond networks (Fengel, Wegener 2011a; Klemm et al. 2005).

2.1.1 Crystal structure

The crystal structure of native cellulose (cellulose I) can be described by a monoclinic unit cell containing two cellulose chains in a parallel orientation with a twofold screw axis. Cellulose I is known to be present in two different crystalline forms, I_α and I_β, which can be found alongside each other. Cellulose also occurs in other crystal allomorphs (cellulose II, III and IV) of which cellulose II is the most stable structure of technical relevance. It is obtained from cellulose I

by alkali treatment with aqueous sodium hydroxide (mercerization) or by dissolution of cellulose and subsequent regeneration, as is done in the formation of fibres and films. The difference between the polarity of cellulose I and cellulose II chains, which are represented in Figure 2-2, has been investigated in the literature (O'Sullivan 1997; Okano, Sarko 1985), where the latter one was found to be antiparallel in contrast to the parallel confirmation of cellulose I chains. What characterizes this monoclinic crystal structure with two antiparallel chains in the unit cell is the specific cell geometry with a modified H-bonding system (Klemm et al. 2005).

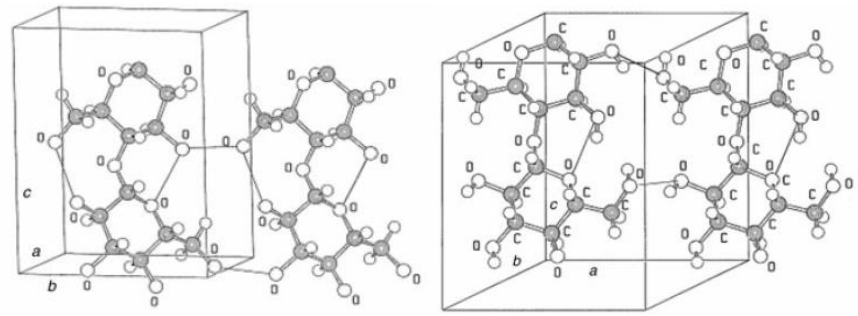


Figure 2-2. Crystal structures of cellulose I_β (left) and cellulose II (right) (Klemm et al. 2005)

Cellulose II and alkali-cellulose

Cellulose I is converted into cellulose II by alkalization which is of considerable importance to commercial-scale cellulose production as a method for increasing the reactivity of subsequent reactions as well as for the mercerization of cotton. Treatment of cellulose with alkali solution results in its swelling to various extents depending on the concentration of alkali, the temperature and mechanical load. At low concentration only the large pores in the cellulose structure are occupied, whereas with the increased concentration the smaller cations, such as Na^+, can more easily advance into smaller pores resulting in highest swelling. Therefore, with the increased alkali concentration the OH-groups become more accessible for water. The most complete transformation of

cellulose I to cellulose II is reached with NaOH due to the favourable diameter of Na^+ cations able to widen the smallest pores, while other alkalis result only in partial or no transformation. Thus, cellulose I is converted into various crystalline alkali forms, each with a different crystal structure and variable NaOH and water content. All forms are then converted into crystalline hydrocellulose during washing, and subsequently to cellulose II through drying (Fengel, Wegener 2011a; Klemm et al. 2005). Based on the fact that Na-cellulose I cannot be reconverted to cellulose I, it was deduced that Na-cellulose I possesses an antiparallel arrangement in the form of the same chain polarity which is thought to exist in cellulose II. This may explain how cellulose I (parallel) can be converted to cellulose II (antiparallel) without solubilizing the cellulose; however, this transformation is not yet entirely understood (O'Sullivan 1997).

2.1.2 Hydrogen bonding

The ultrastructure of native cellulose is determined by the presence of covalent and hydrogen bonds, as well as van der Waals forces. The formation of supramolecular structures is, however, due to the functional groups able to interact with each other. The functional groups of the cellulose chains are hydroxyl groups, three of them being linked to each glucose unit. Numerous OH-groups on the surfaces of cellulose chains are not only responsible for the supramolecular structure, but also for the chemical and physical behaviour of cellulose. OH-groups are able to interact with each other or with O-, N- and S-groups forming the hydrogen bond (H-bond). The hydroxyl groups of cellulose molecules are able to form two types of hydrogen bonds depending on their site at the glucose units. Intramolecular linkages – H-bonds between OH-groups of adjacent glucose units in the same cellulose molecule - give certain stiffness to the single chains, whereas intermolecular hydrogen bonds between OH-groups of adjacent cellulose molecules are responsible for the formation of supramolecular structures, introducing order or disorder into the system (Fengel, Wegener 2011a; O'Sullivan 1997).

A hydrogen bond is characterized by the following criteria:

- strength of the binding energy, which depends on the charge density and the angle between the atoms linked with each other;
- steric factors which cause an asymmetrical distribution of electrons;
- kinetics of the H-bridges.

Fibrils, the primary structures formed by hydrogen bonds, constitute the wall layers and consequently the whole cell wall. Additionally, the surfaces of isolated wood cells or fibres in the non-dried state are able to form hydrogen bonds with each other. The H-bonds occurring between fibre surfaces result in fibre-fibre bonds that determine mechanical properties of cellulosic materials. Not only do hydrogen bonds exist between cellulose OH-groups but also between cellulose- and water-OH. Depending on the water content, single water molecules or clusters can be linked to the cellulosic surfaces. The water absorption capacity of cellulose depends on the number of free OH-groups not linked with each other (Fengel, Wegener 2011a).

2.1.3 Cellulose in solution

An understanding of the structure of cellulose and its derivatives in solution is of great practical importance for the study of its molecular properties. Cellulose can be dissolved by means of heterogeneous conversion into esters that are soluble in common solvents, such as acetone and ethyl acetate, or ethers mostly soluble in water. Another approach is direct dissolution of cellulose in concentrated acids, such as phosphoric and trifluoroacetic (TFA) acids for the determination of molecular weight; however, dissolving in acids leads to hydrolytic cleavage of the cellulose chains. Therefore, these solutions only provide molecular weights of degradation products (Fengel, Wegener 2011a; Klemm et al. 2005).

For a long time since the middle of 19th century, the copper complexes cuoxam and cuoxene were the only ones known to be capable of dissolving cellulose. Almost a hundred years later, the dissolving powers of other metal complexes, including cobalt ethylene diamine and several similar ones containing metals, such as Ni, Cd and Zn were discovered. Over the past few years some new solvent systems were developed, among which is, for instance, dimethyl formamide (DMF) or dimethyl acetamide (DMAC), and N_2O_4 or NOCl that modifies cellulose to its nitrite form. Other systems known are triethyl amine, urea, dimethyl sulfoxide (DMSO) and phthalic anhydride, as well as DMSO-paraformaldehyde whose dissolution product is monomethylol cellulose (Fengel, Wegener 2011a). In addition to that, the new generation of solvents capable of dissolving cellulose are certain salts in their liquid state that consist exclusively of ions and, therefore, are referred to as ionic liquids (ILs). Some of these salts are 1-butyl-3-methyl- and 1-allyl-3-methylimidazolium chlorides ([bmim]Cl and [amim]Cl), as well as 1-ethyl-3-methylimidazolium acetate ([emim][OAc]) and 1-ethyl-3-methylimidazolium methylphosphonate ([emim][MeHPO3]) (Kilpeläinen et al. 2007; Leskinen et al. 2011; Cho, Gross & Chu 2011).

Dissolution of cellulose starts with dissociation of the fibrous and fibrillar structures and should result in complete disintegration into individual molecules with unchanged chain lengths. The degradation of the supramolecular structures occurs by swelling and insertion of chemical groups which cleave the intermolecular linkages and solvate the single molecules (Fengel, Wegener 2011a). Unlike dissolution of cellulose fibres in metal complexes where strong swelling in thickness occurs, in non-aqueous solvents containing amines and polar organic solvents the dissolving process proceeds gradually, beginning at the fibre surface (Philipp, Schleicher & Wagenknecht 1977). In the first phase of dissolving, the particles are still in close contact with each other due to the high concentration. Gel particles enclosing supramolecular structures of cellulose in various dimensions are also detached, and they may survive a further dilution subsequently disturbing viscosimetric measurements. Disregarding the

remaining gel particles, contact between the molecules relaxes with the increase in the distance from the place of dissolving, as well as with the dissolution progress.

The behaviour of macromolecules depends very much on their concentration in solution. According to the scheme of Schurz (1977), the initial dense molecular network containing associated particles is dispersed during dilution to an entangled network and isolated associations (Fig. 2-3). Solution states of cellulose derivatives depend on the type of solvent, polymer concentration, chain length distribution and degree of cellulose substitution. An ideal separation into individual molecules occurs only in highly diluted solutions, i.e. for cellulose within 0.05%. Even in a solution of 0.1% cellulose nitrate (DP 6000) the molecules still form a network (Fengel, Wegener 2011a; Klemm et al. 2005; Schurz 1977).

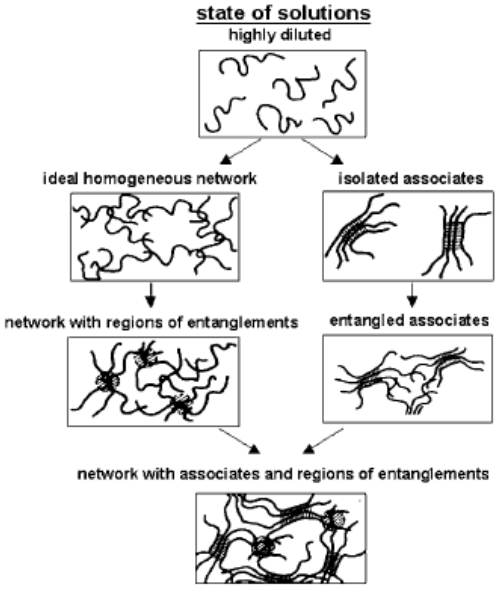


Figure 2-3. Potential dissolution states of cellulose (Klemm et al. 2005)

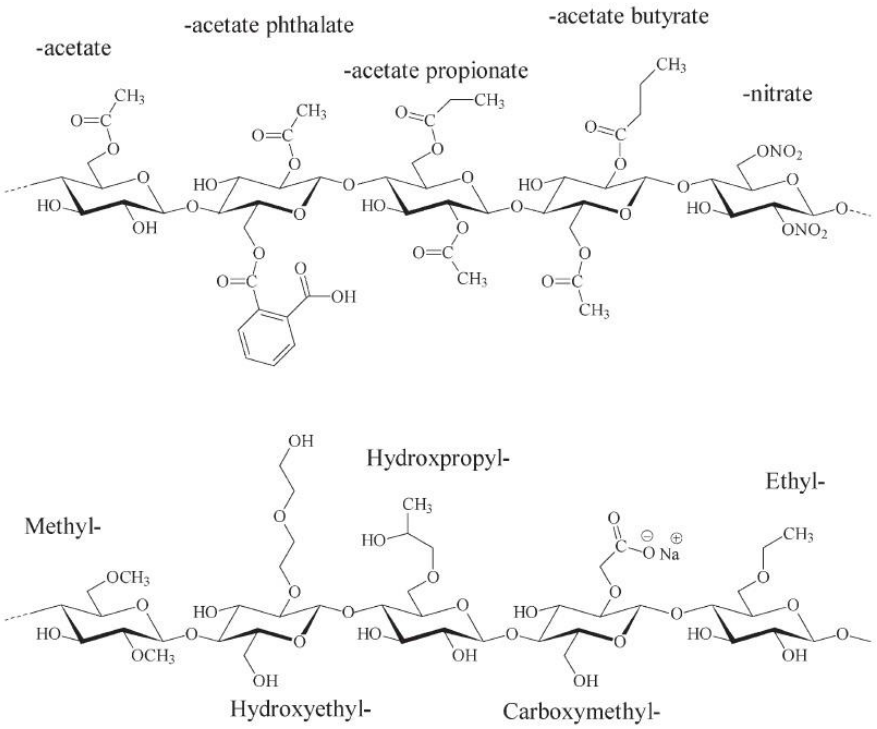
As concluded by Brown (1966), the cellulose molecules are assumed to have flexibility similar to that of other polymers in solution. In cellulose derivatives, the substituents have little steric influence on the expansion of the

macromolecules, but they determine the chain composition through the polymer-solvent interaction.

2.2 Chemical functionalization of cellulose

The aim of chemical functionalization is to adjust the properties of macromolecule for different purposes, particularly, as a chemical feedstock for production of cellulose derivatives for a variety of applications. It plays a dominant role in improving the overall utilization of cellulosic polymers. Chemical functionalization of cellulose includes reactions of hydroxyl groups, such as esterification, etherification, intermolecular crosslinking reactions and macrocellulosic free radical reactions, particularly in the formation of graft cellulose copolymers to increase the usefulness of cellulose by altering its properties (Varshney, Naithani 2011).

This subchapter presents an overview of the most conventional methods of cellulose derivatization, namely esterification and etherification, where the latter one is investigated from the carboxymethylation point of view due to its direct relevance to the current research work. Functionalization by means of the chemical conjugation of carboxylic groups applied in this study is also overviewed.



Scheme 2-1. Commercially produced cellulose esters (top) and ethers (bottom) (Heinze 2009)

2.2.1 Esterification of cellulose

Esters of cellulose are long known derivatives of the polysaccharide. Hydroxyl groups present in cellulose play an important role in its chemical conversion. The esterification reaction of primary and secondary hydroxyl groups of cellulose does not differ from that of other alcohols. Three OH-groups present at each glucose unit make the formation of mono-, di- or triesters possible. Mutual linkages of the OH-groups by hydrogen bonds within the supramolecular structure of cellulose are partially or totally cleaved during esterification. The introduced ester groups push the cellulose chains apart, thus causing strong changes in the structure or even destroying it. Splitting of the molecule chain competes with the catalyzed esterification, but can be controlled under appropriate conditions. Formation of cellulose esters is theoretically possible with all inorganic and organic acids. The most important of them are cellulose nitrate, cellulose xanthate and cellulose acetate because of their technological

and commercial utilization. Cellulose sulphate, nitrite, phosphate, tricarbanilate, and some esters with higher carbonic acids have also gained importance (Fengel, Wegener 2011b; Heinze, Liebert 2001).

Cellulose nitrate

Cellulose nitrate is produced commercially for various purposes, such as for plastics, lacquers, adhesives or explosives, depending on the nitration level. Aqueous nitric acid with concentration below 75% results in poor esterification of cellulose. Therefore, to achieve higher level of nitration, application of acid mixtures (e. g., HNO_3-H_2SO_4, HNO_3-H_3PO_4, HNO_3-acetic anhydride) is needed. The cellulosic starting materials are chemical grade pulp or preliminarily disaggregated cotton linters. Different qualities of the resulting cellulose nitrate are determined by the nitrating acid, treatment time and temperature. Cellulose nitrate is separated from the residual nitrating acid in centrifuges. Next, washing and cooking with water, or pre-stabilization, is applied to remove adhering acid. Stabilization occurs by subsequent cooking under pressure that reduces the chain length of the molecules and equalizes the distribution of the NO_2-groups (Fengel, Wegener 2011b).

Cellulose xanthate

Cellulose xanthate is an important intermediate in the manufacturing of regenerated cellulose. The first step in its production is the treatment of alkali cellulose with carbon disulphide that results in sodium cellulose xanthate. Xanthation of cellulose proceeds rapidly in the less ordered regions, whereas in the ordered regions, the xanthation follows at a low velocity with CS_2, which was absorbed in the structural elements during the first step. An increase in temperature accelerates the reaction, and the amount of absorbed CS_2 decreases. Xanthation is known to occur between a solid phase and an intermediate liquid phase of hydrated carbon disulphide. If the content of free

NaOH in the alkali cellulose is reduced by neutralization, for instance, with gaseous SO_2, the degree of esterification increases, and the formation of by-products is suppressed (Fengel, Wegener 2011b). Cellulose xanthation is additionally overviewed in the section 2.4 of the current chapter.

Cellulose acetate

Cellulose acetate is used for manufacturing of plastics, lacquers, films and fibres. Its technical properties are determined by the degree of substitution (DS) that is responsible for its compatibility with plasticizers and lacquer resins, as well as for solubility in solvents. Another criterion is the degree of polymerization expressed by viscosity, which influences the mechanical behaviour of the products. The acetylation reaction requires the presence of a catalyst; sulphuric acid and perchloric acid have proved to be the most effective, and they are the only ones applied in technical processes. The starting materials for the technical production of cellulose acetate are cotton linters or chemical grade pulp. After activation with acetic acid, the cellulose is treated with glacial acetic acid, which is a solvent for triacetate, a surplus of acetic anhydride and sulphuric acid in a cooled kneader. The degradation of the cellulose chains is regulated by controlling the temperature to obtain the desired viscosity. The reaction is finished when the cellulose acetate is completely dissolved in the reaction medium. After cleaning the solution by filtration, the cellulose acetate is precipitated by the addition of dilute acetic acid with vigorous stirring. Finally, the precipitate is washed with water, centrifuged or pressed for removing the water, and then dried (Fengel, Wegener 2011b).

2.2.2 Etherification of cellulose: carboxymethyl ethers

Cellulose etherification is an important branch in commercial cellulose derivatization, whose industrial production started in the early 1920s in Germany. Presently, the level of the worldwide industrial manufacture of

cellulose ethers is about half a million tons annually, with carboxymethyl cellulose, methyl cellulose and hydroxyethyl cellulose comprising the most of this amount (Klemm et al. 1998).

Amongst numerous highly engineered ionic cellulosic derivatives, CMC is still a dominant and widely applied ionic ether with the annual production volumes of 300 000 tons worldwide. The classical process of CMC preparation implies etherification performed by reaction of alkali cellulose, which is obtained by steeping with aqueous NaOH of 20-30w% concentration and subsequent pressing, with sodium monochloracetate or monochloacetic acid. However, this original two-step process has been widely substituted by a one-step slurry process for large-scale production, where cellulose is suspended in isopropanol or t-butanol and water mixture (inert liquids like benzene or acetone have been mentioned as components as well), treated with aqueous sodium hydroxide solution and then converted with monochloroacetic acid or its sodium salt (Klemm et al. 1998). The latter method and its potential for the variation of structural features of CMC has been extensively studied, and the findings state that changes in concentration or the prolongation of reaction times only influence the overall DS_{CMC}, but cause no effect on the distribution of the substituents within the AGU or along the polymer backbone (Heinze, Liebert 2001; Heinze, Pfeiffer 1999).

In order to control the distribution of the functional groups, a new synthesis path was established. Block-like CMCs can be created by the so-called concept of reactive structure fractions, which is based on a stepwise etherification using aqueous NaOH solutions of comparatively low concentrations. Carboxymethylation is, thereby, mainly achieved selectively in the non-crystalline areas of the cellulose structure. This connection exploits the fact that the activation of cellulose by aqueous NaOH is dependent on the concentration of a base and on the lateral dimensions of the ordered areas. Therefore, with

appropriate concentration of a base, non-crystalline chain segments can react in a block-like manner (Klemm et al. 2005; Heinze, Liebert 2001).

A different route for the synthesis of CMC with new structural features is the activation of cellulose by dissolution in N,N-dimethylacetamide (DMA)/LiCl and a subsequent addition of solid water-free NaOH particles, which initiates a phase separation under gel formation. At the solution-particle interface, active cellulose is regenerated in the chain segments with sodium monochloracetate yielding CMCs with DS values as high as 2.2 in one reaction step (Klemm et al. 2005; Heinze, Liebert 2001).

2.2.3 Chemical conjugation of polysaccharides

The hydroxyl groups of polysaccharides may be activated by certain compounds forming intermediate reactive derivatives that contain good leaving groups for nucleophilic substitution. Reaction of these activated hydroxyls with nucleophiles, such as amines, results in stable covalent bonds between the carbohydrate and the amine-containing molecule. Among activating agents that can be employed for this purpose are carbonyl diimidazole, certain chloroformate derivatives, tresyland tosyl chloride, cyanogen bromide, divinylsulphone, cyanuric chloride, disuccinimidyl carbonate and various bis-epoxide compounds. Such activation steps are frequently done in non-aqueous solutions (i.e., dry dioxane, acetone, dimethylformamide (DMF) and more) to prevent hydrolysis of the active species that occurs faster than hydroxyl group modification due to the relative high abundance of water molecules compared to the amount of carbohydrate OH-groups present. In some cases, even if modification does occur, the resultant bond may be unstable. For instance, N-hydroxysuccinimide (NHS) esters can react with hydroxyls to form ester linkages, which are unstable to hydrolysis. Although many pure polysaccharides can tolerate these organic environments, many biological glycoconjugates cannot. Thus, these methods are suitable for activating pure polysaccharides, such as

dextran, cellulose, agarose and other carbohydrates, but are not appropriate for modifying sugar residues on glycoproteins (Hermanson 1996).

2.2.3.1 Carbodiimide-assisted amide formation

Chemical groups that specifically react with carboxylic acids are limited in variety. In aqueous solutions, the carboxylate functional group displays rather low nucleophilicity. For this reason, it is unreactive with the great majority of bioconjugate reagents that couple through a nucleophilic addition process. Several important chemistries that allow conjugation through a carboxylate group have, however, been developed (Hermanson 1996).

Carbodiimides are probably the most popular type of zero-length cross-linkers used to achieve the formation of amide linkages between a carboxylate molecule and an amine. These compounds mediate the conjugation of two molecules by forming a bond containing no additional atoms. From the two basic types of carbodiimides – water-soluble and water-insoluble – the first ones are the most common choice for biochemical conjugations, as most macromolecules of biological origin are soluble in aqueous buffer solutions. Regardless of the type of carbodiimide, the reaction proceeds by the formation of an intermediate o-acylisourea that is highly reactive and short-lived in aqueous environments. Water-insoluble carbodiimides, by contrast, are used frequently in peptide synthesis and other conjugations, involving molecules soluble only in organic solvents (Hermanson 1996).

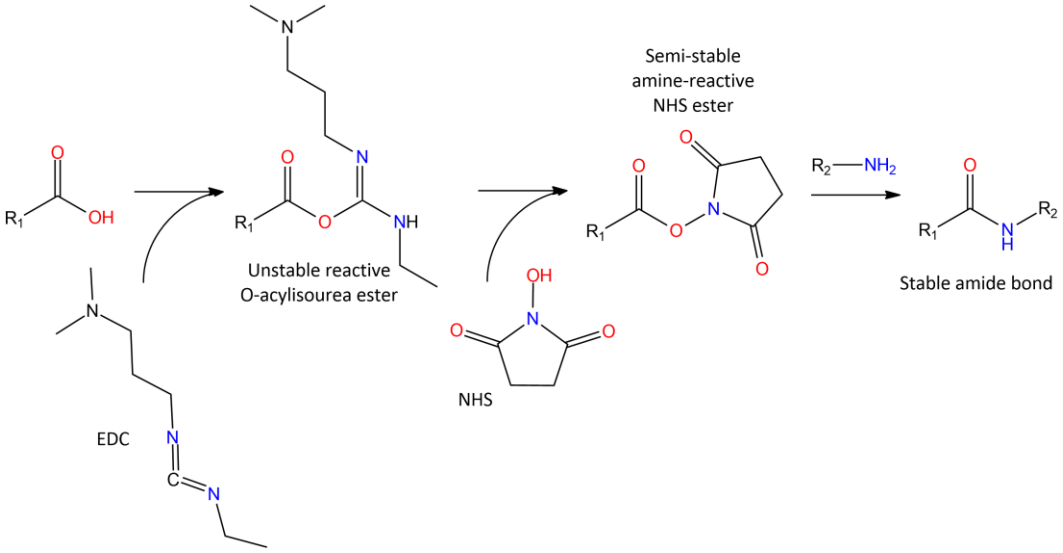
1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) is the only water-soluble activator, which can be added to the reaction directly without prior organic solvent dissolution. Excess reagent and the isourea formed as the by-product of the cross-linking reaction are both water-soluble and may be easily removed by dialysis or gel filtration. The reagent is, however, labile in the presence of water and, therefore, if the urea intermediate does not react in seconds with amine

containing molecules, the N-substituted urea will be released into the reaction medium.

2.2.3.2 NHS esters

N-hydroxysuccinimide (NHS) ester is the most common in activation chemistry for creating reactive acylating agents. Today, the great majority of commercially available amine-reactive cross-linking or modification reagents utilize NHS esters. NHS ester may be formed by the reaction of a carboxylate with NHS in the presence of a carbodiimide. To prepare stable NHS ester derivatives, the activation reaction must be done in non-aqueous conditions using water-insoluble carbodiimides or condensing agents, such as N,N'-dicyclohexylcarbodiimide (DCC) (Hermanson 1996).

NHS esters also may be formed *in situ* to react immediately with target molecules in aqueous reaction media. Using the water-soluble carbodiimide EDC, a carboxylate-containing molecule can be transformed into an active ester functional group by reaction in the presence of NHS or sulfo-NHS (N-hydroxysulfosuccinimide). Unlike NHS esters that are relatively water-insoluble and must be first dissolved in an organic solvent before being added to aqueous solutions, sulfo-NHS esters are relatively water-soluble and long-lived as well as hydrolyze slower in water. In the presence of amine nucleophiles that can attack at the electron-deficient carbonyl of the active ester, the sulfo-NHS group rapidly leaves, creating a stable amide linkage with the amine compound. The advantage of adding NHS esters to EDC reactions, as demonstrated in Scheme 2-2, is that it prevents the hydrolysis failure of the active intermediate and increases its stability, causing it to react ultimately with the attacking amine (Staros, Wright & Swingle 1986). If the target amine does not find the active carboxylate before it hydrolyzes, the desired coupling cannot occur. By adjusting the molar ratio of cross-linker to the target molecules, the level of modification and conjugation may be controlled to create an optimal product.



Scheme 2-2. Proposed mechanism for the EDC/NHS-assisted coupling between carboxylic acids and amines

2.3 Surface modification of cellulose fibres by polyelectrolyte adsorption

Cellulose fibres have been used for centuries in traditional industries including papermaking and textile, medicine and analytical applications. The presence of the OH-groups on the surface of cellulosic materials enables possibilities for their modification. For example, polymer adsorption on cellulose surfaces has been widely utilized in papermaking process. The adsorption is typically driven by the charge difference between negatively charged cellulose and cationic polymers. A recent addition to this has concerned the surface modification aimed at extending applications of cellulosic materials to novel fields. It is done by introducing intelligent properties by means of grafting of new chemical groups at the surface or within limited depth, as discussed in this subchapter.

Adsorption generally refers to the adhesion of atoms, ions or molecules from gas, liquid or dissolved solid to a surface. It can be either irreversible if covalent chemical bonds are formed, or reversible if formation of only physical attachment occurs. Adsorption depends substantially on various factors,

including ionic strength, especially if it is driven by electrostatic interactions, medium, polyelectrolyte dosage, charge density and molecular weight (Wågberg 2000).

Compared to monomeric ions, the entropy content of a given mass of dissolved polymer is quite low. Each repeating unit, due to its connection to adjacent units, has considerable constraints on its freedom of movement at any moment in time. As a consequence, the system can lose only a moderate amount of entropy attributable to transfer a dissolved polymer from the solution to the surface. Meanwhile, any monomeric species that may be displaced from a surface, due to polymer adsorption, can positively contribute to the entropy change (Hubbe, Rojas 2008). However, “high affinity” adsorption (Wågberg 2000) of cationic polyelectrolytes onto cellulosic fibres can be expected since an enthalpic term, due to charge-charge attraction, is added to the entropic force. Moreover, strong electrostatic interactions between cellulosic surfaces and polyelectrolytes can be expected due to a multiplicity of potential points of adsorption.

Irreversible adsorption of various polysaccharides, including water soluble hemicelluloses and certain cellulose derivatives onto cellulose surface has been considered a non-destructive method for introduction of new conjugation sites on cellulosic materials. The adsorption of cellulose-like ionic polysaccharides, such as CMC on cellulose kraft pulps, nanofibrillar cellulose and textile fibres has been demonstrated in the literature (Laine et al. 2000; Fras Zemljič et al. 2006; Filpponen et al. 2012; Junka et al. 2014; Hensley, Inks 1959), where anionically charged carboxymethyl cellulose was found to adsorb onto cellulose despite the expected electrostatic repulsion towards cellulose. The mechanism behind this non-ionic attachment has remained partly unravelled, but it has been proposed that the cellulose-like backbone is the most dominant factor that causes polysaccharides to adhere to cellulose (Mishima et al. 1998). However, an electrolyte is needed to diminish the repulsive electrostatic interactions, allowing CMC to adhere irreversibly on cellulose. Laine et al. (2000) achieved quantitative

results for the adsorption of negatively charged CMC onto different kraft pulps. The study proposes that CMC, the anionic polyelectrolyte, acts as a "bifunctional" material with charged and substantive functions, and uncharged segments are assumed to provide a strong tendency for chain-like hydrogen bonding to cellulosic surfaces. Several factors, such as pH, ionic strength, temperature, concentration and molecular weight have been found to influence the adsorption of CMC on cellulose. In general, the dominating factors for the CMC adsorption are the molecular weight, the degree of substitution of CMC and the ionic strength of the solution.

2.4 Regenerated cellulose: viscose

The first step in viscose manufacturing is isolation of cellulose from the wood. For this purpose, the logs are de-barked and then chipped into small pieces. The pulping process that is carried out next is designed to remove as much lignin, hemicelluloses and other extractable materials as possible, whilst avoiding degradation of the cellulose, though some controlled degradation is allowed in order to produce cellulose of the desired DP. The next step is bleaching to remove any residual lignin, which can be carried out by a process that avoids the use of chlorine and employs a three-stage process instead: firstly, using liquid oxygen, then ozone and, finally, hydrogen peroxide. The final product consisting of 90–92% of α-cellulose and residual hemicelluloses is formed into perfectly white sheets. Of the original wood stock, about 40% is extracted as cellulose for use in the viscose production, 10–11% as secondary products, such as furfural, acetic acid and xylose, and the remaining materials are incinerated, producing steam and electricity (Mather, Wardman 2011).

Viscose is produced from dissolved cellulose: the cellulose sheets are shredded and their moisture content is adjusted to 50%. The wood pulp flakes are then steeped in 18% sodium hydroxide solution, where the sheets swell as the alkali

diffuses into them and reacts chemically with the hydroxyl groups of the cellulose to form alkali cellulose:

$$\mathrm{Cell-OH + NaOH \rightarrow Cell-O^{-}Na^{+} + H_2O}$$

The alkali cellulose is separated from the steeping lye by pressing and then shredded to obtain a bulky, reactive product. The shredded crumbs are next aged in air at ambient temperature for up to 24 hours, where oxidative degradation of the cellulose chains occurs, resulting in a decrease of molar mass. The alkali celluloses are then fed into churns and rotated under vacuum, where carbon disulphide (CS_2) is gradually introduced. The carbon disulphide reacts with the soda cellulose to form sodium cellulose xanthate, which is bright orange in colour (Mather, Wardman 2011):

$$\mathrm{Cell-O^{-}Na^{+} + CS_2 \rightarrow Cell-O-CS-S^{-}Na^{+}}$$

Side reactions can also occur:

$$\mathrm{3CS_2 + 6NaOH \rightarrow 2Na_2CS_3 + Na_2CO_3 + 3H_2O}$$

$$\mathrm{Na_2CS_3 + 6NaOH \rightarrow 3Na_2S + Na_2CO_3 + 3H_2O}$$

These side reactions are more significant at higher temperatures, whereas lower temperatures require longer time to achieve complete xanthation. As a compromise a temperature between 25–37°C is used, with times of 30–90 minutes. The sodium cellulose xanthate is dissolved in 1–2% sodium hydroxide solution at 8–12°C to give an orange-brown spinning solution, the viscose "dope", which is then aged for a further 1–3 days until it reaches the correct viscosity and "ripening index" for extrusion. This period also allows the CS_2 to become evenly distributed throughout the cellulose. Before extrusion, the viscose dope is filtered to remove any small undissolved solids which might clog the spinneret holes, and any air bubbles which would disrupt the flow of polymer

through the spinneret are removed by deaeration under vacuum. At this stage, additives may be also added to the dope. For instance, there are surface active agents that improve spinning performance, and white finely dispersed titanium dioxide pigment that transforms the standard “bright” fibres to dull or matt when required (Mather, Wardman 2011).

The fibres are produced by wet spinning. The spinneret, made of a gold–platinum alloy with thousands of small holes, is submerged in a coagulating bath containing 10% sulphuric acid, 18% sodium sulphate and 1% zinc sulphate. During the extrusion of the sodium cellulose xanthate, the cellulose molecules near to and in contact with the walls of the orifices of the spinneret experience a drag and align to a greater extent than the molecules in the centre. The composition of the coagulating bath is important (sometimes magnesium sulphate is added) since it controls the rate of generation and quality of the viscose. Typically, the fibres develop with a “skin-core” structure, whereby the orientation of the outer layer of the fibres is greater than that of the inner layer (Mather, Wardman 2011).

The chemical reactions taking place in the coagulation bath can be represented as:

$$2\mathrm{Cell} - \mathrm{O} - \mathrm{CS} - \mathrm{SNa} + \mathrm{H_2SO_4} \rightarrow 2\mathrm{Cell} - \mathrm{OH} + 2\mathrm{CS_2} + \mathrm{Na_2SO_4}$$

The presence of zinc sulphate favours fibre strength and results in serrated cross-section. The divalent zinc ion is thought to form a weak cross-link between adjacent cellulose xanthate anions:

$$\mathrm{Cell} - \mathrm{O} - \mathrm{CS} - \mathrm{S^-} \cdots \mathrm{Zn^{2+}} \cdots {}^{-}\mathrm{S} - \mathrm{CS} - \mathrm{O} - \mathrm{Cell}$$

This results in a retardation of the regeneration process. During the extrusion process, the zinc ions diffuse only slowly into the fibres and only into the outer layer, in contrast to the much more mobile hydrogen ion that penetrates

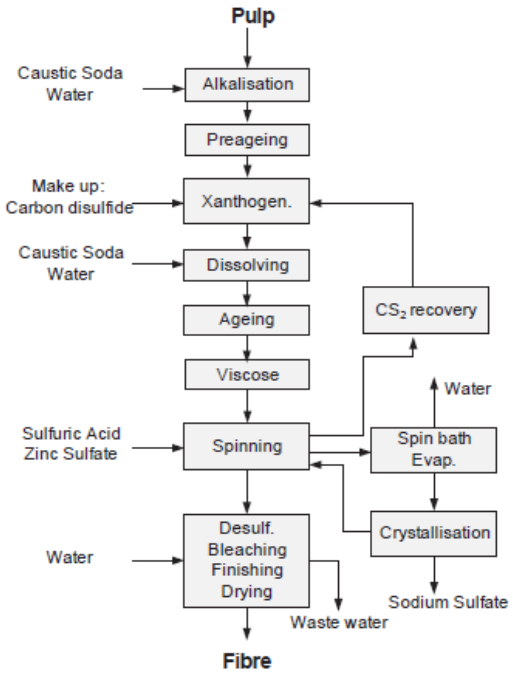
throughout the fibre. The regeneration of the cellulose therefore occurs more slowly on the outer layer of the fibre, and because of the aligning effect of the spinneret described above, causes a more uniform, aligned arrangement of the cellulose molecules. As the core shrinks on regeneration of cellulose, the skin also contracts and becomes wrinkled, giving the fibres their characteristic jagged cross-sectional shape and striations along their length.

During the extrusion stage the products of the side reactions formed in the xanthation stage, Na_2CS_3 and Na_2S, can react with the sulphuric acid present in the coagulation bath to give CS_2, H_2S and Na_2SO_4:

$$Na_2CS_3 + H_2SO_4 \rightarrow CS_2 + H_2S + Na_2SO_4$$

$$Na_2S + H_2SO_4 \rightarrow H_2S + Na_2SO_4$$

The resultant CS_2 is recovered back to the xanthation stage. After extrusion, thousands of individual fibre filaments are fed as a tow out of the bath into a second hot water bath via a pair of Godet rollers. The second roller rotates faster than the first one in order to induce a strength-enhancing stretch into the fibres. The tow is usually cut into staple lengths of around 40 mm, formed into a fleece and finally thoroughly washed and dried.



Scheme 2-3. Schematic representation of the viscose manufacturing process (Mather, Wardman 2011)

Viscose fibres, like cotton fibres, comprise almost pure cellulose and, therefore, show very similar chemical properties in terms of their reactions with acids, alkalis and oxidizing agents. Viscose differs quite substantially in its molecular structure, however, and has a less complex morphology than cotton. To begin with, the number of glucose units in wood cellulose is only about 1000 compared with up to 10 000 in cotton cellulose. During the manufacture of viscose, when the cellulose is converted into sodium cellulose xanthate and aged, some scission of the cellulose molecules occurs due to oxidative depolymerization, so that the degree of polymerization of the final viscose fibres is only around 270. This considerably lower molecular mass of viscose cellulose manifests itself in a much lower strength than cotton fibres, and with behaviour opposite to that of cotton, the strength of viscose fibres decreases when they are wet. Viscose fibres swell considerably in water, with their diameter increasing by approximately 35–40%. They have a water retention value of about 85–90%, a value more than double to that of cotton. Naturally, since the extrusion process can be controlled, the

diameter of the viscose fibres produced can be perfectly uniform and also the fibres can be produced with any degree of lustre.

The properties of regular viscose fibres are given in Table 2-1. The main attributes of viscose fibres are their silk-like handle and shiny, lustrous appearance. Viscose is used extensively for both apparel and non-apparel applications. In apparel, it is widely used for linings because it is smooth and shiny so that garments, such as jackets or overcoats slip easily over garments worn underneath. Other applications are those where its natural absorbency is useful, such as lingerie, blouses, dresses, and skirts. One interesting variant of viscose is Viloft with a special flat cross-sectional shape which creates air pockets for excellent thermal insulation, quick moisture transport and breathability (Figure 2-4). Some other examples are Galaxy with a trilobal cross-section that delivers a fibre with 30% higher level of absorbency than other viscose fibres, and Danufil – widely used in medical and hygiene products, textile, specialty paper and packaging manufacturing (Kelheim Fibres GmbH 2014).

Table 2-1. Properties of viscose fibres

Fibre length	Usually ~40 mm, but can be varied
Fineness	20 µm
Specific gravity	1.52
Tenacity	25-30 cN tex^{-1} dry, 18-20 cN tex^{-1} wet
Elongation at break	Fairly extensible; stretches by ~15%
Elastic recovery	Does not recover well from stretching
Resilience	Very good
Moisture regain	12-13%
Reaction to heat	No melting point, thus is very heat-resistant; yellows with a hot iron
Sunlight	Gradual strength loss on exposure
Launder ability	Best washed at 40 °C; dry-cleaned but not tumble-dried; slow drying and easy creasing

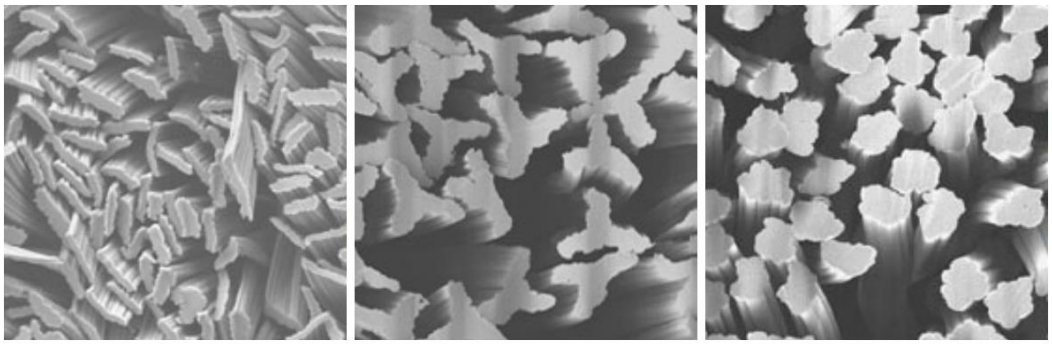


Figure 2-4. Cross-sections of Viloft (left), Galaxy (middle) and Danufil (right) fibres (Kelheim Fibres GmbH 2014)

2.5 Introduction to click chemistry

Over the recent years robust and quick reactions have been developed under the concept of "click chemistry". This approach was first termed in 2001 by Sharpless and co-workers (Kolb, Finn & Sharpless 2001) to describe bond-forming reactions applied for rapid assembly of molecules with functions of interest. Several efficient reactions which are capable of producing a wide catalogue of functional synthetic molecules and organic materials have been grouped accordingly under this term. Click transformations are easy to perform, give very high yields while generating very little of harmless and easily removable by-products, and are unaffected by the nature of the clickable groups. The process characteristics include insensitivity to oxygen or water, mild solventless (or aqueous) conditions, regiospecificity and stereospecificity, as well as readily available starting materials and reagents. Click reactions achieve these characteristics due to high thermodynamic force, proceeding rapidly to completion and being highly selective (Such et al. 2012). Cu(I)-catalyzed 1,3-dipolar cycloaddition of alkynes and azides (CuAAC) reaction is the premier example that has received the most attention and is reviewed in more details within this subchapter. Its applications extend to the synthesis of biomedical libraries, dendrimer preparation, synthesis of functional block copolymers, synthesis of uniformly structured hydrogels, derivatization of cellular surfaces, the *in situ* preparation of enzyme inhibitors,

and even more (Kolb, Finn & Sharpless 2001; Koschella, Hartlieb & Heinze 2011). A non-catalytic variation of the 1,3-dipolar azide-alkyne cycloaddition was also developed for functionalization of polysaccharides by addition of difluorocyclooctynes (DIFO), which finds interesting applications in the biochemical area due to the removal of copper toxicity. This reaction, achieved by increasing the reactivity of the alkyne by introducing a ring strain, is often referred to as a Strain Promoted Alkyne-Azide Cycloaddition (SPAAC) (Elchinger et al. 2011).

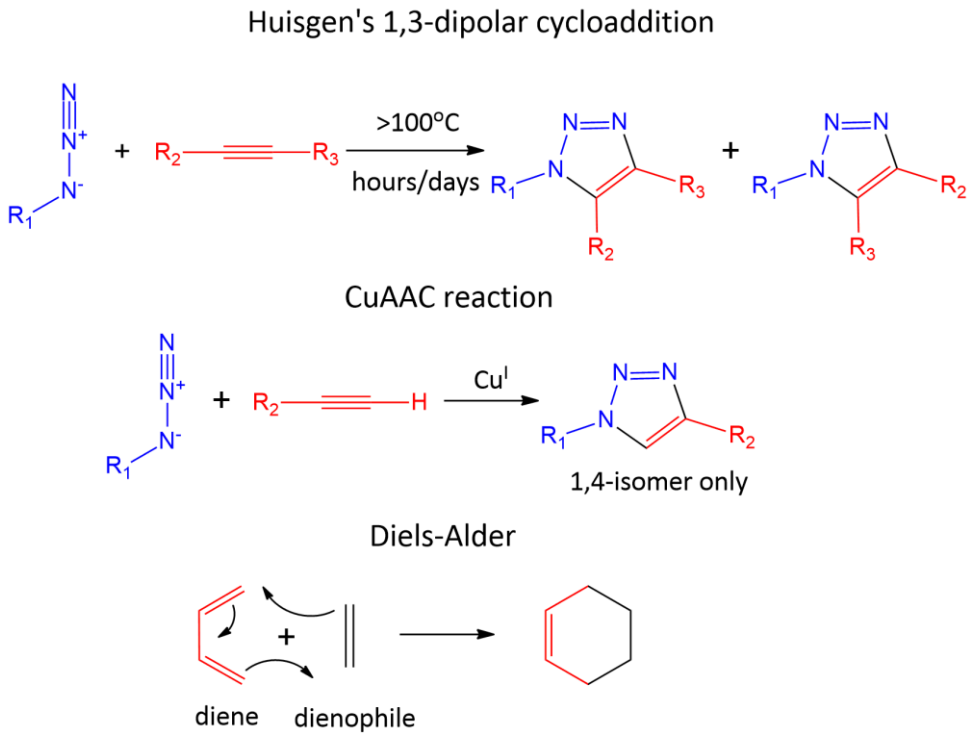


Figure 2-5. Most common click chemistry reactions

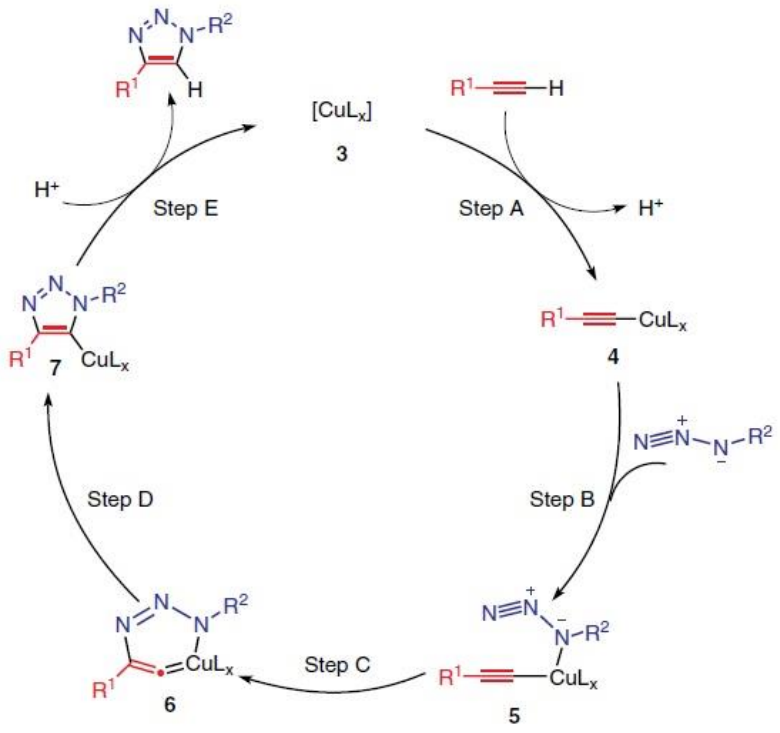
Amongst other popular “click” reactions are also thiol-ene radical and thiol Michael addition reactions. Highly efficient reactions of thiols with reactive carbon-carbon double bonds, or simply “enes”, have been known since early 1900s. During the last century, two thiol reactions, as illustrated in Figure 2-5, emerged: thiol-ene free-radical addition to electron-rich/electron-poor carbon-carbon double bonds, and the catalyzed thiol Michael addition to electron-deficient carbon-carbon double bonds (Hoyle, Bowman 2010). The reactions of thiols with enes, whether proceeding by a radical (thiol-ene reaction) or anionic chain (thiol Michael addition), carry many of the attributes of click reactions that include achieving quantitative yields, requiring only small concentrations of relatively benign catalysts, having rapid reaction rates with reactions occurring in either bulk or environmentally benign solvents and vast availability of a wide range of both thiols and enes. These properties make the thiol–ene chemistry amenable to applications ranging from high performance protective polymer networks to processes that are important in the optical, biomedical, sensing, and bioorganic modification fields. Accordingly, both the thiol-ene radical and thiol Michael addition reactions are now routinely referred to in the literature as thiol click reactions (Hoyle, Bowman 2010; Hoyle, Lowe & Bowman 2010).

Another click reaction recognized since 1928 is the [4+2] Diels-Alder (DA) cycloaddition between a diene and dienophile. The Diels-Alder and the opposite retro Diels-Alder (rDA) reactions can be controlled by temperature to form the cyclized product and noncyclized starting materials, respectively. Being attractive for a range of applications, this reaction also produces thermoreversible adducts which enables fabrication of materials incorporated with “self-healing” pathways. This feature of the cycloadducts has long been recognized by polymer scientists and a wide variety of mendable materials have been fabricated using the diene-dienophile-based crosslinking of polymers. Recent years have witnessed utilization of the Diels–Alder cycloaddition reaction to design and obtain thermoreversible discrete macromolecular compounds like dendrimers and dendronized polymers (Nicolaou et al. 2002; Sanyal 2010).

2.5.1 Copper-catalyzed azide-alkyne cycloaddition reaction (CuAAC)

Mechanism of CuAAC

The CuAAC reaction occurs between an organic azide and a terminal alkyne in the presence of Cu(I) to form regioselectively 1,4-disubstituted 1,2,3-triazoles. Copper catalysts significantly affect the mechanism and outcome of the reaction, converting it to a sequence of discreet steps culminating in the formation of a 5-triazolyl copper intermediate (Scheme 2-4). Next, this intermediate coordinates the organic azide, and, subsequently, the nucleophilic carbon of the copper(I) acetylide reacts with the electrophilic terminal nitrogen on the azide. Finally, this metal cycle undergoes ring contraction and subsequent dissociation of the product to regenerate the catalyst (Such et al. 2012; Hein, Fokin 2010). Cu(I) catalysis increases the rate of reaction up to 10^7 times faster than such of the non-catalyzed reaction, meaning that it proceeds efficiently at and even below the room temperature (Bock, Hiemstra & van Maarseveen 2006). One of the most remarkable features of the CuAAC reaction is its ability to proceed in a variety of protic and aprotic solvents, including water, as well as to tolerate a wide range of pH values. The reaction is unaffected by most of the organic and inorganic functional groups that eliminates the need for protecting group chemistry (Bock, Hiemstra & van Maarseveen 2006; Hein, Fokin 2010).



Scheme 2-4. Proposed catalytic cycle of CuAAC reaction (Wu, Fokin 2007)

The copper(I) catalyst

The formation of triazoles from azides and terminal alkynes catalyzed by copper(I) is an extraordinarily robust reaction, which could be performed under a wide variety of conditions and with almost any source of solvated Cu(I). In order to facilitate the reaction, it is of great importance to keep the Cu(I) concentration at its maximum. A variety of copper catalysts can be used for the CuAAC reaction provided that some Cu(I) species is generated. Sources include Cu(I) salts (iodide, bromide, chloride or acetate), coordination complexes, such as $[Cu(CH_3CN)_4]PF_6$ and $[Cu(CH_3CN)_4]OTf$, as well as Cu(I) and Cu(0) compounds, the surface of which forms the required Cu(I) species (Such et al. 2012; Bock, Hiemstra & van Maarseveen 2006; Hein, Fokin 2010; Liang, Astruc 2011).

The most common conditions are the aqueous conditions employing $CuSO_4$ and sodium ascorbate as a reducing agent. A large difference is observed between the dependence of base in the application of $CuSO_4$ and Cu(I)-halide salts. While

the catalytically active Cu(I) species is directly generated by reduction with ascorbate and immediately forms Cu-acetylides, the CuI and CuBr salts require at least an amine base or high temperature to form the Cu-acetylide complexes (Meldal, Tornøe 2008). However, Hein and Fokin (2010) discourage the use of copper iodide due to the ability of iodide anion to act as a bridging ligand for the metal, resulting in the formation of polynuclear acetylide complexes that interfere with the productive catalytic cycle by hampering the catalyst. Additionally, under certain conditions copper iodide may result in the formation of 1-iodialkynes and, consequently, 5-iodotriazoles. Comparing to the iodide, the inhibitory effect of chloride is less pronounced. Cuprous bromide and acetate, as well as the sulphate from *in situ* reduction of $CuSO_4$, are favoured for reactions performed in aqueous solvents, whereas for organic reactions the cuprous acetate salt is a decent choice (Hein, Fokin 2010).

Among the three most common oxidation states of copper (0, +1 and +2), Cu(I) is the least thermodynamically stable. Therefore, cuprous ions can be oxidized to catalytically inactive Cu(II), or disproportionate to a mixture of Cu(II) and Cu(0). When present in significant amounts, Cu(II) can facilitate the oxidative alkyne coupling processes which may result in the formation of undesired byproducts while impairing triazole formation. Therefore, when a copper(I) catalyst is used directly, exclusion of oxygen may be required to prevent complications. As an alternative to oxygen-free conditions, ascorbate, a mild reducing agent, can be used. Its combination with a copper(II) salt, such as the readily available and stable copper(II) sulphate pentahydrate or copper(II) acetate, is a practical solution as well as the method of choice for preparative synthesis of 1,2,3-triazoles (Hein, Fokin 2010; Wu, Fokin 2007; Rostovtsev et al. 2002).

3 EXPERIMENTAL PART

3.1 Scope of the study

The surface of the viscose fibres was modified by the irreversible adsorption of functionalized CMC and subsequent covalent attachment of "clickable" functional groups onto the pre-modified fibres by means of the CuAAC click reaction. The aim of the experimental part was to evaluate the adsorption efficiency of CMC and its "clickable" derivatives onto viscose in different reaction conditions, as well as study the chemical and mechanical stabilities of the modification method applied. CMC functionalization was analyzed with spectroscopic techniques and elemental analysis. The effect of various reaction conditions on adsorption was studied and quantified by means of the phenol-sulphuric acid method which determines the amount of adsorbed carbohydrates. Chemical and mechanical stabilities of modified CMC and fibres were studied by the alkaline and wet frictional treatments, respectively.

3.2 Materials and methods

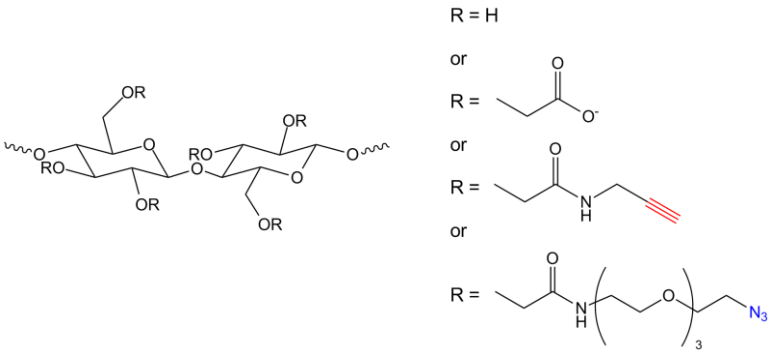
3.2.1 Chemicals and solutions

Washed, never-dried, non-woven and unfinished staple viscose fibres (dtex 1.7) supplied by Kelheim Fibres GmbH were modified with functionalized carboxymethyl cellulose (M_w 250 000, DS 0.7) manufactured by Sigma-Aldrich (Finland). All the CMC used in experimenting was purified via dialysis, dried to its solid form and subsequently functionalized with 11-azido-3,6,9-trioxaundecan-1-amine and propargylamine in 2-(N-morpholino)ethane sulfonic acid (MES) buffer solution with addition of coupling reagents, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and N-hydroxysiccinimide (NHS), all

obtained from Sigma-Aldrich. All solutions for analyses were prepared with MilliQ ultrapure water. For phenol-sulphuric acid method, acid of 95-97% grade was used.

3.2.2 Synthesis of functionalized CMC

Synthesis of the modified CMCs, as illustrated in Scheme 3-1, was performed according to Filpponen et al. (2012). CMC-Na salt was dissolved in ultrapure MilliQ water (consistency 4 $g \cdot l^{-1}$) by stirring overnight, subsequently dialyzed against deionized (DI) water for three days (SpectraPor dialysis membrane, M_wCO 12000-14000 $g \cdot mol^{-1}$) and recovered by lyophilization. A 400 mg amount of purified CMC was dissolved and mixed overnight in 100 ml of the MES buffer solution (50 mM, pH 5), which was prepared by dissolution of 9.76 g of MES in MilliQ water, adjustment of pH with 1M NaOH and dilution to the final volume of 1 l. Next, coupling reagents were added to the CMC mixture: 400 mg of EDC and 900 mg of NHS followed by addition of either 150 µl of propargylamine or 450 µl of 11-azido-3,6,9-trioxaundecan-1-amine for alkyne and azide functionalizations, respectively. After mixing overnight, solutions were dialyzed against DI water (M_wCO 6000-8000 $g \cdot mol^{-1}$) for three days, and purified CMC-derivatives were recovered by lyophilization as aforementioned. Degree of substitution (DS) of achieved CMC-adducts was then estimated according to elemental analysis results. The presence of characteristic amide, alkyne and azide functional groups was evaluated with Fourier transform infrared spectroscopy (FTIR).



Scheme 3-1. Molecular structures of cellulose, CMC, alkyne- and azide-functionalized CMCs

3.2.3 Fibre pretreatment and CMC adsorption

Prior to adsorption experiments, fibres were washed with an excess of 0.1M HCl for 30 minutes to remove metal ions and convert ionizable groups on the fibres into their acid form. After this treatment, the fibres were washed with DI water to a conductivity level below 5 $\mu S \cdot cm^{-1}$. Next, CMC was adsorbed onto the fibres at various conditions according to the following procedure:

- The CMC was dissolved overnight in $CaCl_2$ electrolyte solution of concentrations equal to 0.025M, 0.05M and 0.1M;
- Pretreated wet fibres of around 1 g of dry equivalent were then added to $CaCl_2$ solution containing dissolved CMC, and adsorption was carried out by mixing for given time at room and elevated temperatures, as summarized in Table 3-1;
- After adsorption, fibres were filtered through a glass filter and subsequently washed few times to ensure that all the unbound CMC was removed from the fibres. Initial and washing filtrates were further utilized for the evaluation of adsorption efficiencies at different conditions by using phenol - sulphuric acid method for the estimation of total adsorbed carbohydrates.

Table 3-1. CMC adsorption conditions

Sample	$CaCl_2$ conc.	T, °C	Time	Consistency
1	0.025M	room T	2h	20mg CMC/1g fibres/50ml $CaCl_2$
2		80		
3		80	15 min	
4	0.05M	room T	2h	
5		80		
6		80	15 min	
7			45 min	
8	0.1M	room T	2h	
9		80		

Modified fibres were additionally analyzed with ultraviolet resonance Raman spectroscopy (UVRR) and X-ray photoelectron spectroscopy (XPS).

3.2.4 Phenol-sulphuric acid method for estimating the adsorbed carbohydrate content

Principle of the method

The determination of carbohydrate concentration in aqueous solutions is an important procedure for the environmental research and insdustries, such as food, petroleum and pharmaceutical, to name few. Simple sugars, oligosaccharides, polysaccharides and their derivatives, including methyl ethers with free or potentially free reducing groups, are dehydrated to hydroxymethyl furfural (HMF) giving an orange-yellow colour when treated with phenol and concentrated sulphuric acid. The reaction is sensitive and the colour is stable. Thus, phenol in the presence of sulphuric acid can be used for the quantitative colorimetric determination of aforementioned compounds (DuBois et al. 1956; Chaplin 1986).

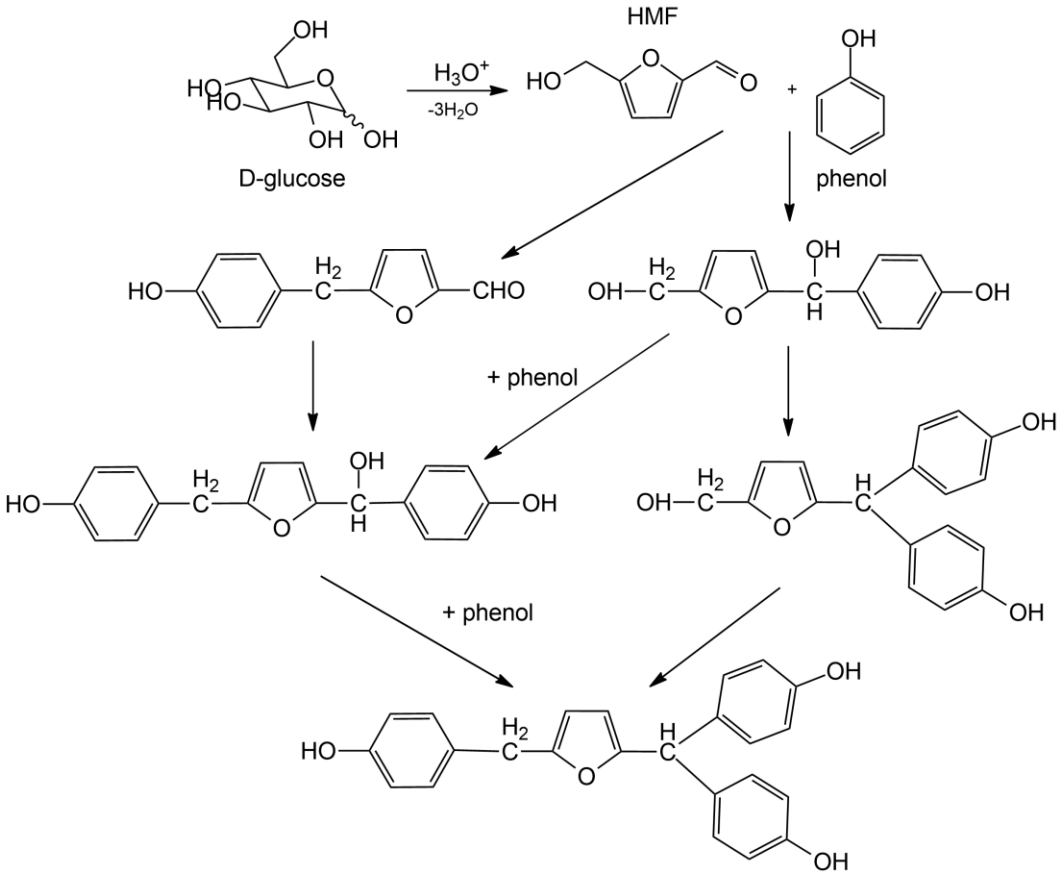


Figure 3-1. Reaction mechanism of phenol-sulphuric acid method (Koch, Pein 1985)

Procedure

First, the set of samples with known concentrations of working standard glucose solution was prepared to obtain a calibration curve. For that, 10 ml of the standard glucose solution (100 mg/100 ml) were diluted to 100 ml of the working standard with distilled water. The set of standards containing 0 (blank), 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard solution were pipetted into separate test tubes and adjusted with distilled water to the total volume of 1 ml. Next, 1 ml of 5% phenol solution was added to each sample followed by immediate addition of 5 ml of concentrated 95-97% sulphuric acid. Sample tubes were shaken and after 10 minutes placed in a water bath at 25-30°C for 20 minutes. The absorbance of each sample was read at wavelength of 490 nm, and the standard curve was obtained based on collected data represented in Appendix A.

Adsorption efficiency, or total adsorbed amount of CMC, at different conditions was evaluated by backwards calculation based on the residual amounts of CMC left in the filtrate solution after adsorption. Filtrates were analyzed according to the same procedure taking 0.2 ml of each per sample. See Appendix A for detailed calculation.

3.2.5 Click reaction on pre-modified fibres

Fluorescent labelling of fibres with dansyl-probe

0.5 g of fibres modified with azido-CMC were suspended in acetone-water solution (50:50 by volume, ml) containing 20 mg of dissolved fluorescent 5-(dimethylamino)-N-(2-propyl)-1-naphthalenesulfon-amide (dansyl alkyne). Next, 1 ml of 7.5% (w/v) copper (II) sulphate pentahydrate solution followed by 1 ml of 1M L-ascorbic acid were added. Control sample was prepared similarly, but excluding a copper catalyst and acidic reductant. Reaction was carried out at room temperature by stirring for 2 hours. Finally, samples were subjected to intensive washing as follows:

- soaked in 60:40 acetone-water solution for at least 15 minutes (2-3 rounds)
- washed with saturated ethylenediaminetetraacetic acid (EDTA) solution to remove copper ions
- washed with DI water

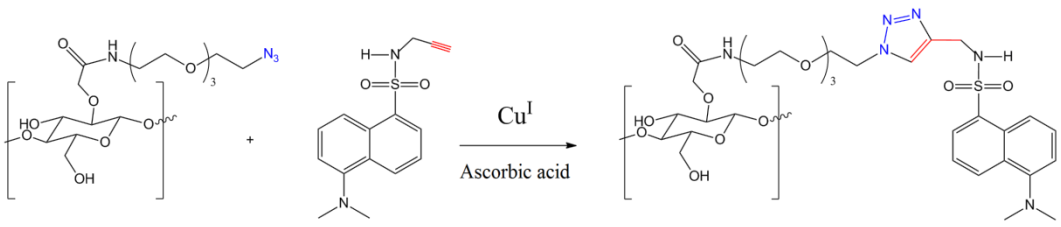


Figure 3-2. Click reaction between azido-CMC and dansyl alkyne

Click reaction between azido-CMC and propargylamine

65 mg of azido-CMC were dissolved in 50 ml of DI water. Next, propargylamine (50 µl), $CuSO_4 \cdot 5H_2O$ (10 mg) and L-ascorbic acid (35 mg) were added. Reaction mixture was stirred for 4 hours and then dialyzed against saturated EDTA solution for 1 day to remove the remaining copper. Finally, the solution was dialyzed against DI water for another day and collected by lyophilization (Filpponen et al. 2012). The so-called "clicked" derivative was then subjected to liquid-state nuclear magnetic resonance (NMR) analysis with the main aim of detecting the triazole ring formation.

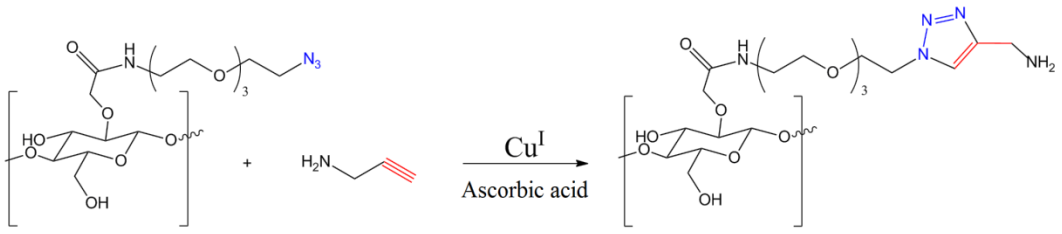


Figure 3-3. Click reaction between azido-CMC and propargylamine

3.2.6 Fourier transform infrared and Raman spectroscopies

The energy of most molecular vibrations corresponds to that of the infrared region of the electromagnetic spectrum. Functional groups have their own characteristic vibration frequencies within well-defined regions of the infrared spectrum range that is between 4000 cm^{-1} and 625 cm^{-1} at the high and low frequency ends, respectively (Williams, Fleming 1989). A technique used to obtain an infrared spectrum of a solid, liquid or gaseous compound is called the Fourier transform infrared spectroscopy (FTIR). FTIR has proved to be one of the most useful methods for characterization of the hydrogen bonds in cellulose, but is also widely used to determine the chemical compositions of native and modified natural fibres.

An IR spectrometer consists of an infrared light source, a sample holder, detector and plotter. To date, the spectrometer employs either a single IR beam or, as in older instruments, it is split into two equal intensity beams. In modern instruments, the background spectrum is obtained and stored first and the sample spectrum together with background – afterwards. The background spectrum is then subtracted by the instrument software resulting in the actual sample spectrum. The method used for sample preparation depends upon the nature of the sample. Liquids are easily examined as films formed when one drop of the liquid is squeezed between two flat sodium chloride plates, which are transparent to IR radiation in the 4000-666 cm^{-1} region. Solids can be examined as solutions, mulls in Nujol (a liquid hydrocarbon), or as potassium bromide discs (Anderson, Bendell & Groundwater 2004).

The CMC derivatives were analyzed with FTIR spectroscopy aiming at detection of azide, alkyne and amide signals. The CMC samples were pressed into potassium bromide discs by finely grounding the solid samples with pure KBr and then pressing the mixture into a disc with a mould and a hydraulic press. The use of KBr eliminates the problem of bands due to the mulling agent, as well as provides better spectra (Williams, Fleming 1989). The spectra were collected with Thermo Nicolet Avatar 360 FT-IR spectrometer.

Another spectroscopic technique that is used to observe low-frequency modes of the system is the Raman spectroscopy. It relies on inelastic scattering (Raman scattering) of monochromatic light usually from a laser in the visible, near infrared or near ultraviolet range. The Raman effect occurs when light interacts with an electron cloud and bonds of a molecule. For the spontaneous Raman effect, which is a form of light scattering, a photon excites the molecule from the ground state to a virtual energy state. The molecule emits a photon as it relaxes, and returns to a different rotational or vibrational state. The difference in energy between the original and the new states leads to a shift in the frequency of the emitted photon away from the excitation wavelength. For a

molecule to exhibit a Raman effect, a change in the molecular polarization potential with respect to the vibrational coordinate is required. The Raman scattering intensity is determined by the amount of the polarizability change. This dependence on the polarizability differs from Infrared spectroscopy where the interaction between the molecule and light is determined by the dipole moment; this contrasting feature allows one to analyze transitions that might not be IR active via Raman spectroscopy (Gardiner 1989).

Pure alkyne- and azide-bearing compounds, unmodified and functionalized CMCs, and all fibre samples were analyzed with the Ranishaw UV-spectrometer. Transmission spectra were collected at 10 mW of laser power and laser beam focus of 30 μm within time intervals of 30 and 60 seconds, depending on the sample.

3.2.7 X-ray photoelectron spectroscopy

X-ray photoelectron spectroscopy (XPS), also known as electron spectroscopy for chemical analysis (ESCA), is a surface-sensitive quantitative spectroscopic technique that measures the elemental composition of a material, as well as chemical and electronic states of the elements that exist within it. The surface chemistry of a material can be analyzed in its as-received state or after some treatment, for example: fracturing, cutting, ion beam etching to remove the surface contamination and more. XPS spectra are obtained by irradiating a material with a beam of X-rays while simultaneously measuring the kinetic energy and number of electrons that escape from the top 0 to 10 nm of the material being analyzed. XPS requires high or ultra-high vacuum conditions, although a current area of development is ambient-pressure XPS, in which samples are analyzed at pressures of a few tens of millibar (Beamson, Briggs 1992; Johansson, Campbell 2004).

XPS measurements were recorded according to Johansson and Campbell (2004) with Kratos Axis Ultra spectrometer. Spectra collection was accomplished using monochromatic Al K_α irradiation at 100 W and a 90° electron take-off angle. Survey scans and the high-resolution regions were recorded with 1 eV step and 160 eV analyzer pass energy, and with 0.1 eV step and 20 eV analyzer pass energy, respectively.

3.2.8 Nuclear magnetic resonance spectroscopy

Nuclear magnetic resonance (NMR) is a physical phenomenon in which nuclei in a magnetic field absorb and re-emit electromagnetic radiation and that allows to observe specific quantum mechanical magnetic properties of the atomic nucleus. Some atomic nuclei have a nuclear spin (*I*) that makes them behave like bar magnets. These nuclear magnets can orient themselves in 2*I*+1 ways in the presence of an applied magnetic field. Nuclei with an odd number of nucleons, of which ^{1}H and ^{13}C are the most important, have spins of 1/2. Thus, they can occupy one of only two orientations – either a low energy orientation aligned with the applied field, or a high energy orientation opposed to the applied field. The difference in energy is given by:

$$\Delta E = h\gamma B_0/2\pi \,,$$

where γ is the magnetogyric ratio, a proportionality constant different for each nucleus that measures the strength of the nuclear magnets, and B_0 is the strength of the magnetic field applied. The number of nuclei in the low (N_α) and high (N_β) energy states differs by an amount determined by the Boltzmann distribution (Williams, Fleming 1989):

$$\frac{N_\beta}{N_\alpha} = exp\left(\frac{-\Delta E}{kT}\right)$$

The resonance frequency is dependent on both the applied field strength and the nature of the nucleus and is given as:

$$v = \gamma B_0 / 2\pi$$

To obtain an NMR spectrum, the sample is dissolved in excess of a fully deuterated solvent (e.g., deuterochloroform, dimethyl sulfoxide-d_6, deuterium oxide), placed into an NMR tube, and then lowered into the probe between the poles of the magnet that generates the magnetic field due to an electric current in coils connected to the probe. The NMR instrument is usually described by the frequency at which protons in the magnetic field absorb energy. A critical parameter in determination of the signal-to-noise and resolution of the NMR data is the strength of the magnetic field used. Another important consideration is the high magnetic field homogeneity that provides longer signal and better NMR data (Anderson, Bendell & Groundwater 2004).

The samples for liquid-state proton NMR were prepared by dissolution of 10 mg of unmodified CMC, its alkyne- and azide-derivatives and "clicked" CMC in 1 ml of deuterium oxide (D_2O) solvent. The spectra were collected with a Varian Inova 500 spectrometer. The number of scans was 32 and the relaxation delay was 5 s. The chemical shifts are reported in ppm relative to residual D_2O (δ 4.80).

3.2.9 Stability of the adsorbed CMC-layer

Chemical stability

Chemical stability of amide bonds formed during functionalization of CMC was tested by treating azido-CMC in alkaline buffer solutions. As the parallel research within the group confirmed, CMC functionalization is not stable under highly alkaline (≥pH 13) or acidic conditions which is most likely due to hydrolysis of amide bonds. Hence, milder conditions were tested. First, 20 mg of the azide-CMC derivative were suspended in 50 ml of alkaline buffer solutions of pH 10, 11

and 12, and mixed for 1.5 hours at room temperature, followed by centrifuging and washing. Next, same procedure was repeated to mimic the in-line washing stage: azido-CMC was treated in pH 7 and pH 11 at 80°C for 15 minutes. The resultant samples were analyzed with FTIR and compared to the original untreated samples.

Mechanical stability and properties

Two samples – fibres modified with azido-CMC and fibres "clicked" with fluorescent dansyl alkyne – were subjected to fibrillation by rotating ball mill. The fibrillation system constituted of 15 ml-volume cylindrical tubes containing stainless steel balls. Aproximately 60 mg of each fibre sample were placed into separate vessels filled with 10 ml of tap water, set vertically into rotating platform and treated for 1 hour at the temperature region of 50-60°C. After the fibrillation treatment, the samples were analyzed with UV Raman spectroscopy. In addition to that, the sample modified with azido-CMC was subjected to the click reaction that would only succeed with azide moiety still present at the fibre surface. The post-treated "clicked" sample was observed under the UV light for detection of remaining fluorescent probe.

4 RESULTS AND DISCUSSION

4.1 CMC functionalization

Elemental analysis

Degree of substitution of CMC-adducts was estimated based on remotely performed C/H/N elemental analysis that confirmed the expected increase in nitrogen content in both azide- and alkyne-functionalized CMCs, yielding the functionalization efficiency of 60% when compared to the initial DS of CMC in terms of carboxymethylation (DS 0.7).

Table 4-1. C/H/N elemental analysis results. Oxygen content assumed to be equal to the residue.

Sample	C%	H%	N%	O%	DS
CMC	35.31	5.04	< 0.05	59.60	0.7
azido-CMC	43.72	6.47	**7.86**	41.95	**0.41**
alkyne-CMC	42.28	6.15	**3.29**	48.28	**0.42**

FTIR and UV Raman spectroscopies

The CMC derivatives and their adsorption onto viscose fibres were also analyzed with spectroscopic techniques aiming at detection of azide, alkyne and amide signals. The stretching frequencies of N-H bonds can sometimes be confused with those of hydrogen bonded O-H frequencies. Due to their much weaker tendency to form hydrogen bonds, N-H absorption is usually sharper. The strong broad band of O-H of water (3600-3200 cm^{-1}), which overlaps with amide N-H stretching band (Table 4-2), is almost always present in KBr discs and was also detected in the azido-CMC sample, as indicated in Figure 4-1. Most carboxylic

acids produce characteristic series of bands in the range of 3000-2500 cm^{-1}. The bands are usually seen as a jagged series on the low frequency side of any C-H absorption which may be present (Williams, Fleming 1989). The presence of carboxylic C=O bonds at the 1760-1690 region indicates the unreacted CMC and, therefore, supports the elemental analysis findings regarding functionalization efficiency.

Table 4-2. Characteristic functional group bands

Functional group	Wavenumber, cm-1
Carboxylic O-H stretch	3000-2500
Carboxylic C=O stretch	1760-1690
Carboxylate ions $–CO_2^-$	1610-1550
Terminal alkyne C-H stretch	3300
Terminal alkyne C≡C stretch	2140-2100 (w)
Azide $–N_3$	2160 – 2120 (s)
Amide C=O stretch	1690 - 1630 (s)
Amide N-H stretch	3700 - 3500 (m)
Amide N-H bend	1650 – 1550 (w)

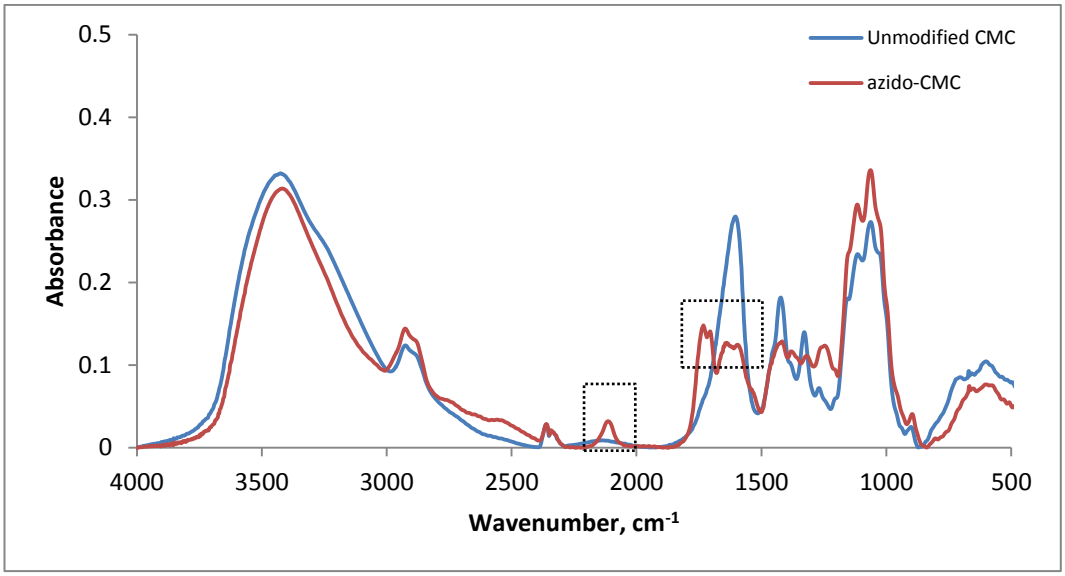


Figure 4-1. FTIR spectra of unmodified and azide-modified CMCs. Weak azide signal at 2100 cm^{-1}; amide N-H bending at 1633 and 1619 cm^{-1}; carboxylic C=O stretching at 1726 and 1700 cm^{-1}. These signals are indicated by framed boxes in the above figure.

As for triple bonds, symmetrical and nearly symmetrical substitution makes the C≡C stretching frequency inactive in the infrared (Williams, Fleming 1989). It is, however, clearly seen on the Raman spectrum at 2120 cm^{-1}, as illustrated in the Figure 4-2 below. The peak absorbance of the unmodified CMC appearing at 1600 cm^{-1} is assigned to the C=O stretching vibration of $–CO_2-$. Clearly, this peak became lower upon conjugation reaction with EDC and NHS and new peaks appeared at 1653 cm^{-1} and 1550 cm^{-1} assigned to C=O stretching and N-H bending vibrations of amide, respectively.

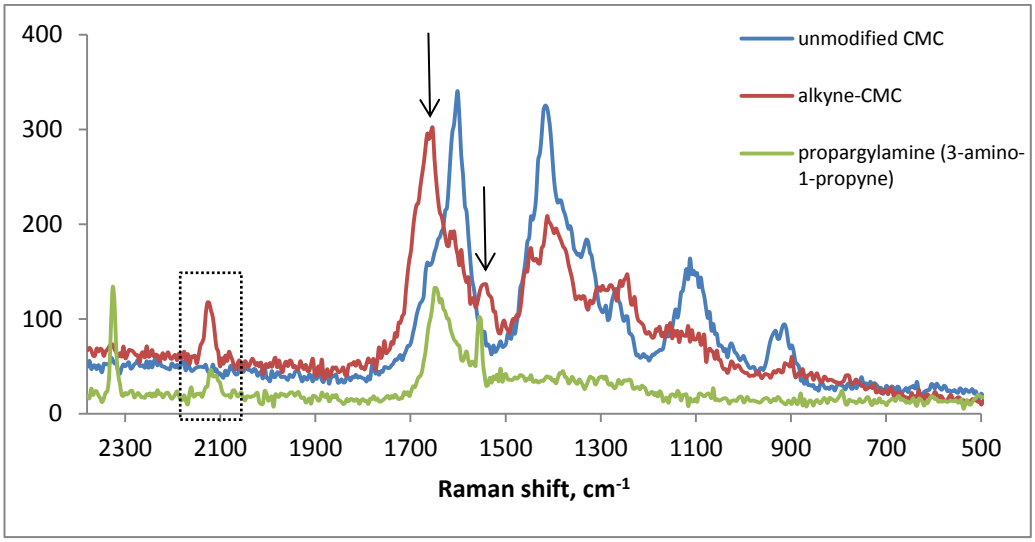


Figure 4-2. UV Raman spectra of unmodified CMC, alkyne-functionalized CMC and the alkyne moiety compound. Alkyne C≡C stretching at 2120; weak amide N-H bending at 1550 cm^{-1} and strong amide C=O stretching at 1653 cm^{-1}. These signals are indicated with arrows.

Liquid-state H-NMR

Unmodified CMC and its azide and alkyne derivatives were subjected to liquid-state proton NMR analysis. Sample preparation was accomplished as described in section 3.2.5 and NMR spectra were obtained by Varian Inova 500 spectrometer. The reference result of unmodified CMC is illustrated in Appendix B (Kono 2013). The obtained spectra proved the expected changes in molecular structures of the CMC-derivatives: the characteristic conjugated terminal alkyne and amide protons were both detected at around 2.8 ppm (Figure 4-3), and conjugated azide and amide protons at 3.65 ppm and 2.8 ppm, respectively (Figure 4-4) (Okoth, Basu 2013).

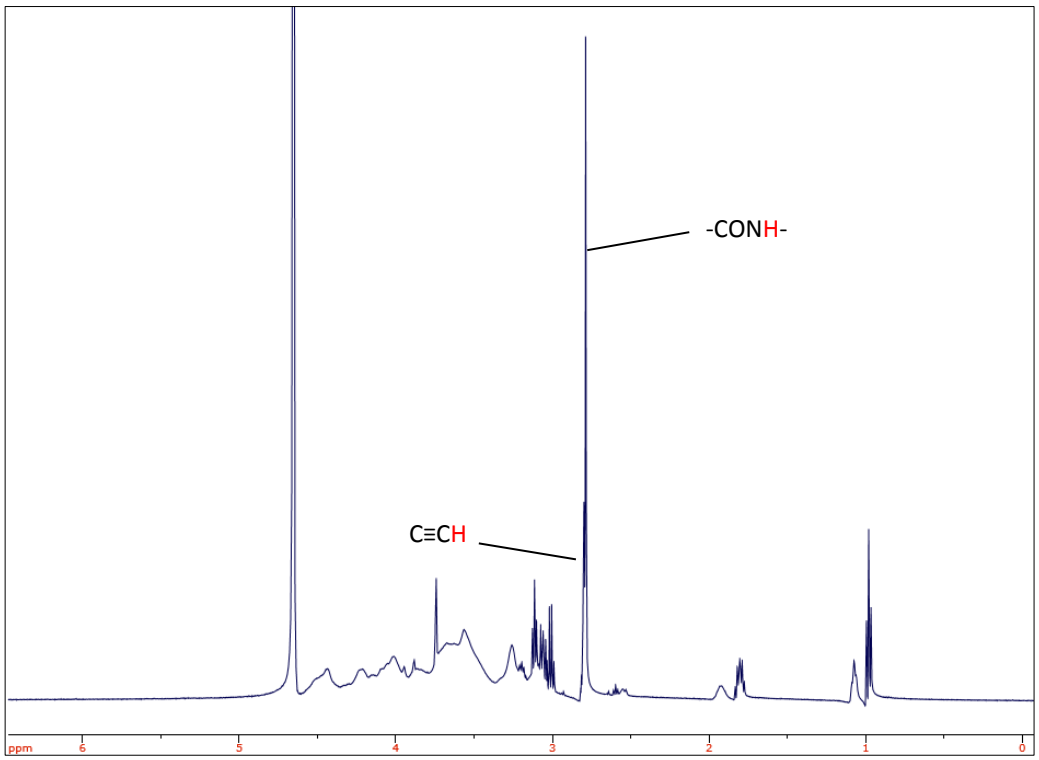


Figure 4-3. H-NMR spectrum of alkyne-modified CMC

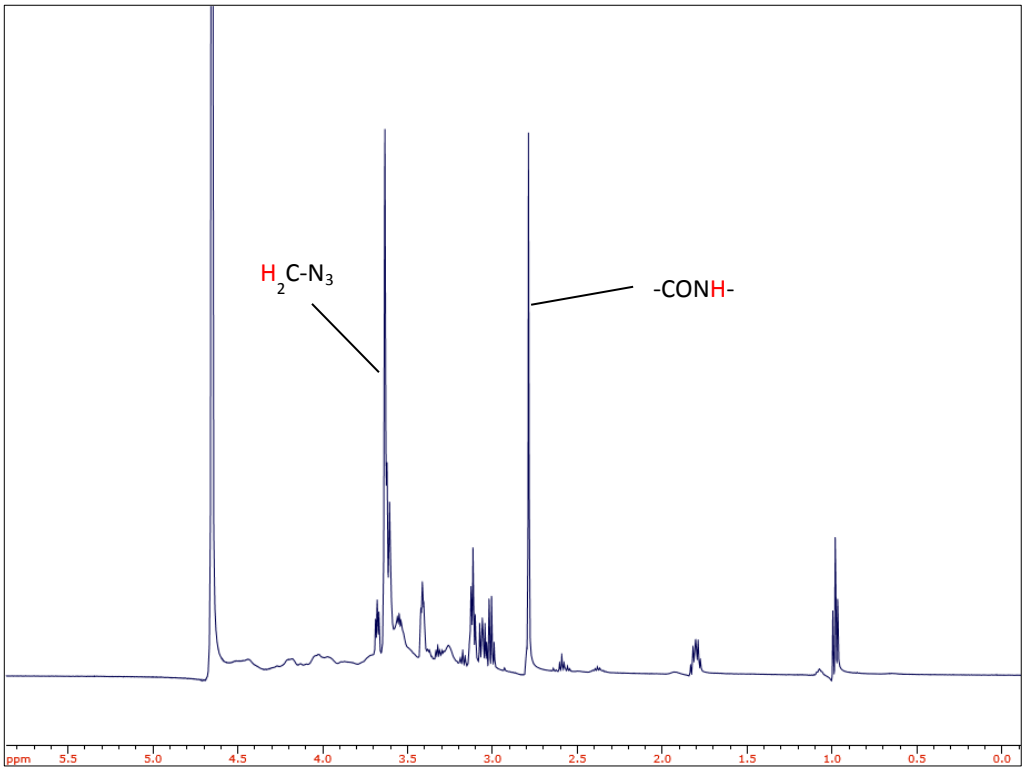


Figure 4-4. H-NMR spectrum of azide-modified CMC

4.2 Adsorption efficiency

Adsorption of CMC onto fibres was carried out in different conditions with varying concentration of $CaCl_2$, temperature and time. The early fibre samples modified with azido- and alkyne-CMCs were also subjected to elemental analysis, but the results did not reveal any change in nitrogen content. This finding is assumed to arouse due to the nitrogen content below the detection limit of elemental analysis. The assumption is based on several factors, such as CMC functionalization efficiency, analyzed fibre pretreatment at conditions resulting in low adsorption levels and the nitrogen ratio to the whole fibre sample that is, apparently, very low.

Adsorption of CMC derivatives onto viscose fibres was evaluated qualitatively by Raman spectroscopy technique which clearly indicated the differences between the unmodified and treated fibres. However, neither azide nor alkyne signals could be detected due to the reasons named in the previous paragraph. The difference between unmodified and treated fibres can be seen from the spectra overlaid in Figures 4-5 and 4-6. The spectra of fibres treated with azido- and alkyne-CMC (spectra in orange line colour) combine peaks appearing for both CMC and its partially functionalized adducts, which supports the statement that azido- and alkyne-CMC did adsorb onto viscose. Another observation is the appearance of the slightly pronounced amide signal at 1653 and 1623 cm^{-1} in the spectra of fibres modified with azido- and alkyne-CMCs, respectively. The aforementioned findings, therefore, prove the fibre modification took place.

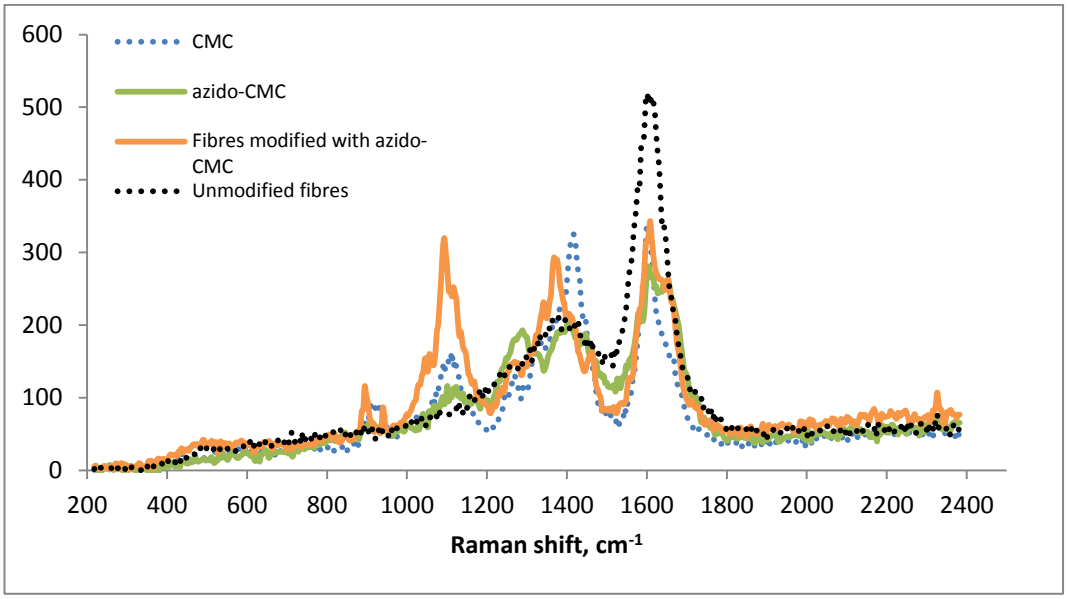


Figure 4-5. UV Raman spectrum of fibres modified with azido-CMC

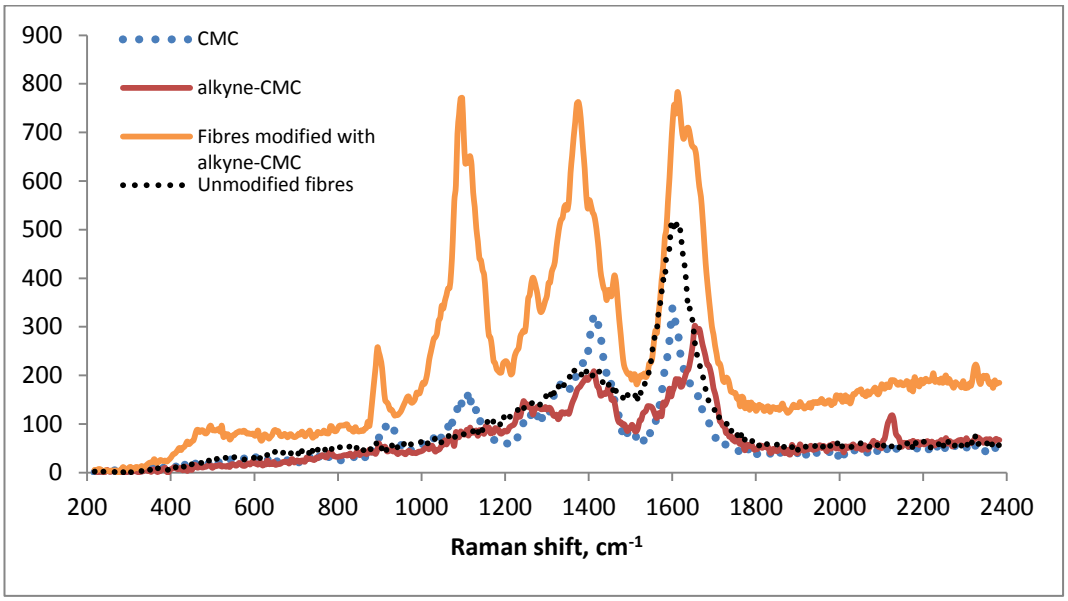


Figure 4-6. UV Raman spectrum of fibres modified with alkyne-CMC

For quantitative evaluation, the phenol - sulphuric acid method for the estimation of total adsorbed carbohydrates was employed. Unmodified CMC was adsorbed onto viscose fibres as explained in section 3.2.3, and resultant filtrates were then exploited for the estimation of residual amounts of the unadsorbed CMC in the filtrate solutions. The trials were repeated few times until satisfactory

quantitative results were obtained. The results are summarized in Table 4-3 below.

Table 4-3. Results of the CMC adsorption onto viscose fibres in different reaction conditions. Adsorption consistency fixed for all trials: 20 mg CMC/1 g fibres/50 ml $CaCl_2$.

Sample	$CaCl_2$ conc.	T, °C	Time	% adsorbed	mg adsorbed
1	0.025M	room T	2h	25.6	5.11
2		80		26.8	5.36
3		80	15 min	27.1	5.42
4	0.05M	room T	2h	33.8	6.77
5		80		30.1	6.02
6		80	15 min	23.0	4.59
7			45 min	28.9	5.78
8	0.1M	room T	2h	23.1	4.62
9		80		34.4	6.88

Adsorption time was reduced from 2 hours to 15 and 45 minutes. By analyzing obtained results there seems to be no clear behavioural pattern taking place. For instance, samples treated in 0.05M $CaCl_2$ at 80°C for aforementioned reaction times resulted in adsorption efficiencies ranging from 23% to 30%. The lowest value refers to the shortest reaction time, whereas results obtained at 45 minutes and 2 hours are nearly the same. 30% efficiency after a 2h-adsorption at these conditions is not too far from around 40% achieved in wood pulp modification studies (Laine et al. 2000). The highest result of 34% was obtained after the 2h-treatment in 0.1M $CaCl_2$ at 80°C. However, in contrast to earlier, similar studies on wood pulp and cotton fibres that identified clearly the effect of different adsorption conditions (Laine et al. 2000; Fras Zemljič et al. 2006), it is difficult to see any specific trend taking place in the viscose case. One possible explanation to behavioural difference is in cellulose crystal structures: pulp and cotton are cellulose I and viscose is cellulose II.

4.3 CuAAC click reaction

Liquid-state H-NMR

The CuAAC click reaction between azide-modified CMC and propargylamine was performed according to procedure described in section 3.2.5, and the liquid state NMR analysis detected the formation of 1,4-disubstituted 1,2,3-triazole ring with a characteristic conjugated proton at 8.1 ppm, as indicated in Figure 4-7.

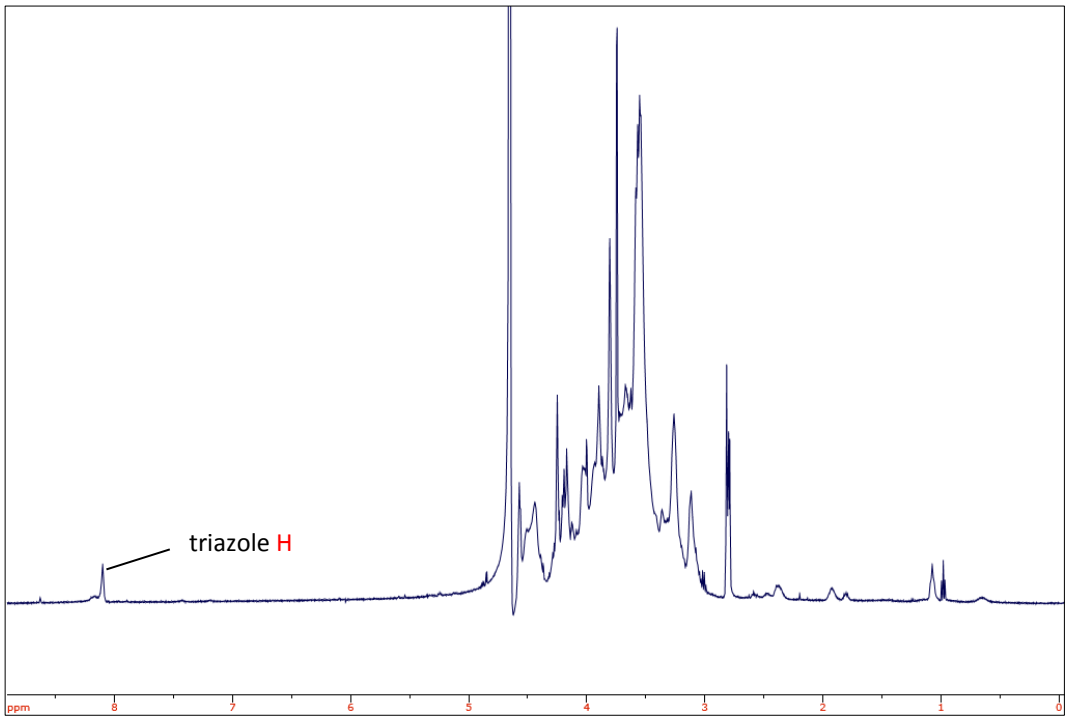


Figure 4-7. Liquid-state H-NMR spectrum of azido-CMC clicked with propargylamine

XPS

Five samples – unmodified viscose fibres, fibres modified with CMC, azido-CMC and alkyne-CMC, as well as the clicked fibres (click reaction with the fluorescent probe) – were analyzed in order to detect the presence of nitrogen on the fibre surface. The XPS experiments were challenging due to the initial viscose charging. Therefore, only selected results out of obtained data range are

presented in Appendix C. Trace of nitrogen was only detected in the clicked sample that is expected to be the most nitrogen-rich out of all modified samples (Figure 4-8). It is thus assumed that nitrogen content in the samples modified with azido- and alkyne-CMCs is below the detection limit of XPS. The choice of adsorption conditions to which analyzed samples were subjected at the time of sample preparation – 0.025M $CaCl_2$, room T, 2 hours, fibre consistency 1 g/100 ml – is likely to explain the achieved somewhat poor results.

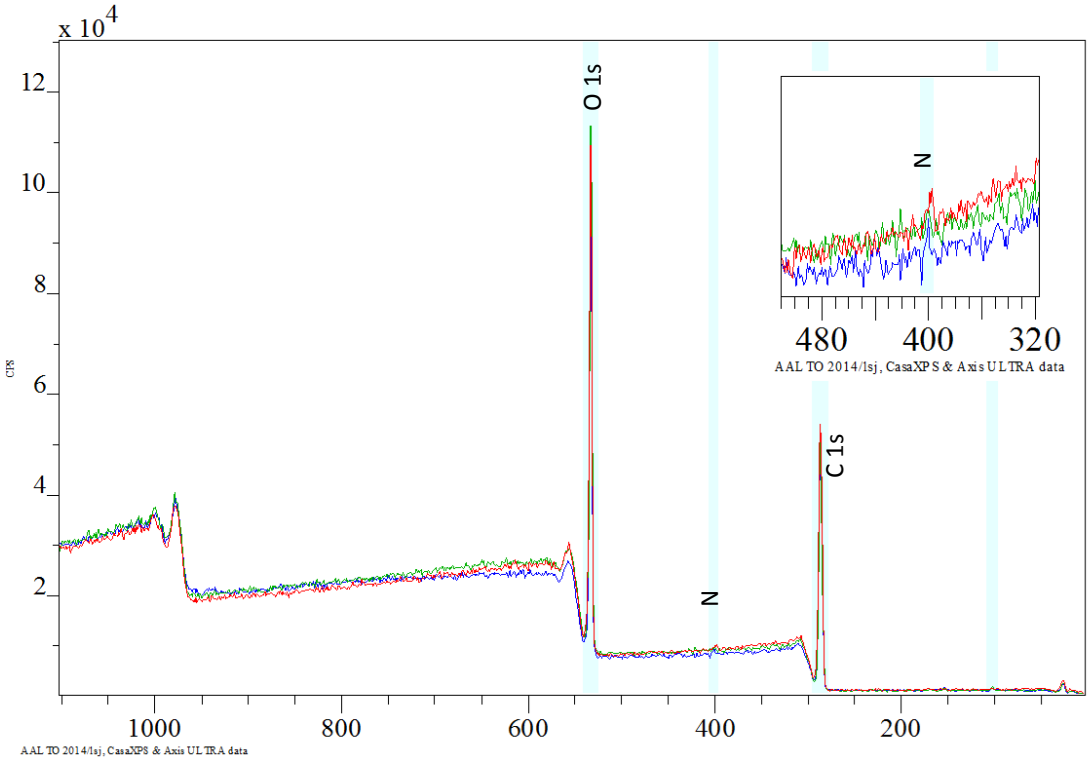


Figure 4-8. XPS spectra of clicked fibres (analyzed in triplicate). The magnified image in the upper right corner demonstrates the presence of trace nitrogen.

Despite the challenging quantitative evaluation, there are clear visual proofs of stable modifications of fibres taking place, as demonstrated in Figure 4-9. This is the final evidence that supports successful click reaction and clearly distinguishes "clicked" fibres (C) from the unmodified ones (A). The control sample B, which was obtained by the click reaction in the absence of the copper catalyst, demonstrates no fluorescence which clearly indicates that the fluorescent dye is

attached to the fibres only via chemical reaction, and not with the physical adsorption.

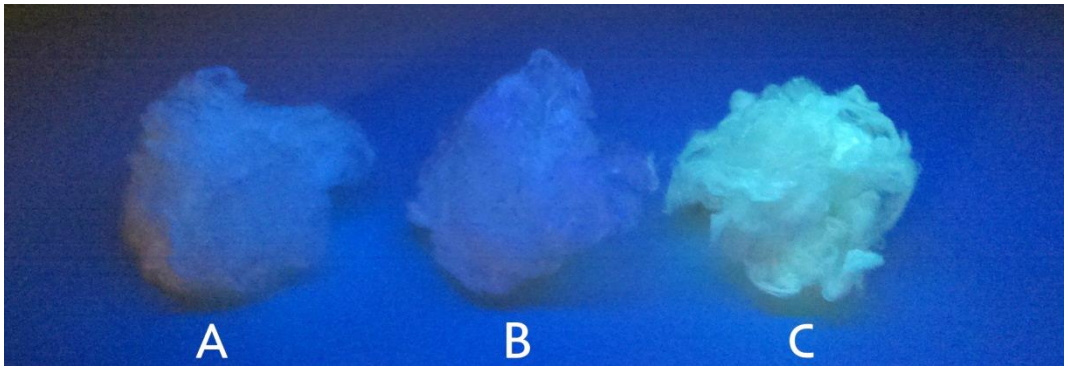


Figure 4-9. Fluorescent labelling of viscose fibres: A – unmodified fibres, B – control sample, C – "clicked" sample under the UV light at the wavelength of 254 nm

4.4 Chemical and mechanical stability

Chemical stability of amide bonds formed during functionalization of CMC was tested by treating azido-CMC in buffer solutions of pH 10, 11 and 12 at room temperature for 1.5 hours, as well as at pH 7 and pH 11 at 80°C for 15 minutes. After the treatments, the characteristic azide signal was still detectable by FTIR which indicates the chemical stability of amide bonds (Figures 4-10, 4-11). Moreover, there is no increase in $–CO_2–$ band at 1700 cm^{-1} that would refer to hydrolysis of amide bonds. It should be noted that the treated samples were thoroughly washed to ensure the removal of any possibly detached azide-bearing amines as a result of amide bond cleavage. Thus, the appearance of azide signal as a cleaved residue is eliminated.

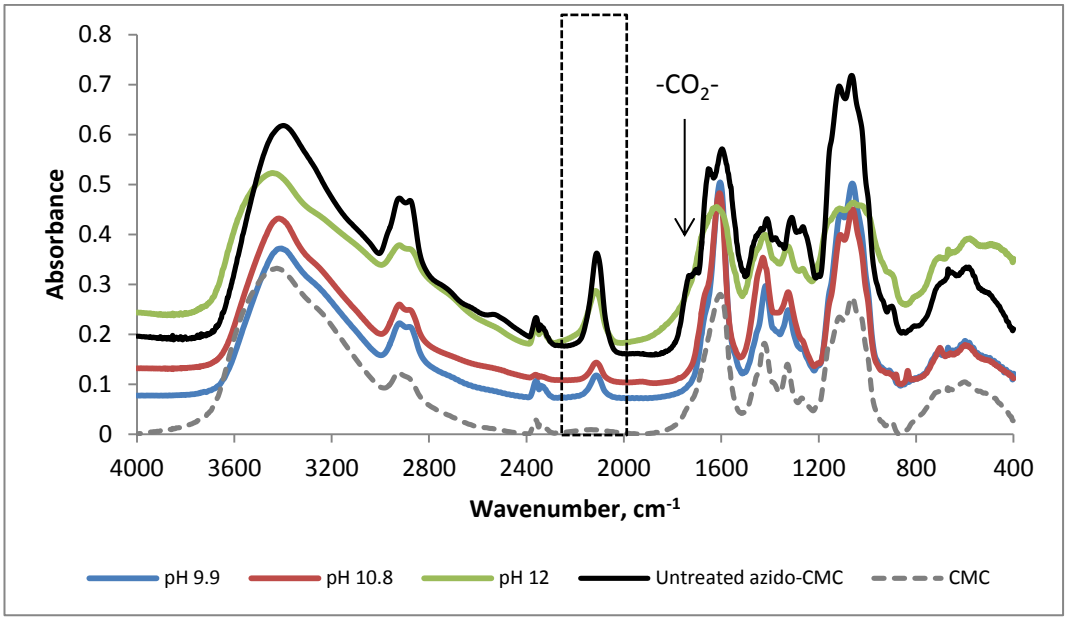


Figure 4-10. FTIR spectra illustrating the chemical stability of azido-CMC after alkaline treatment at room temperature for 1.5 hours. The characteristic azide stretching bands are indicated by the framed box.

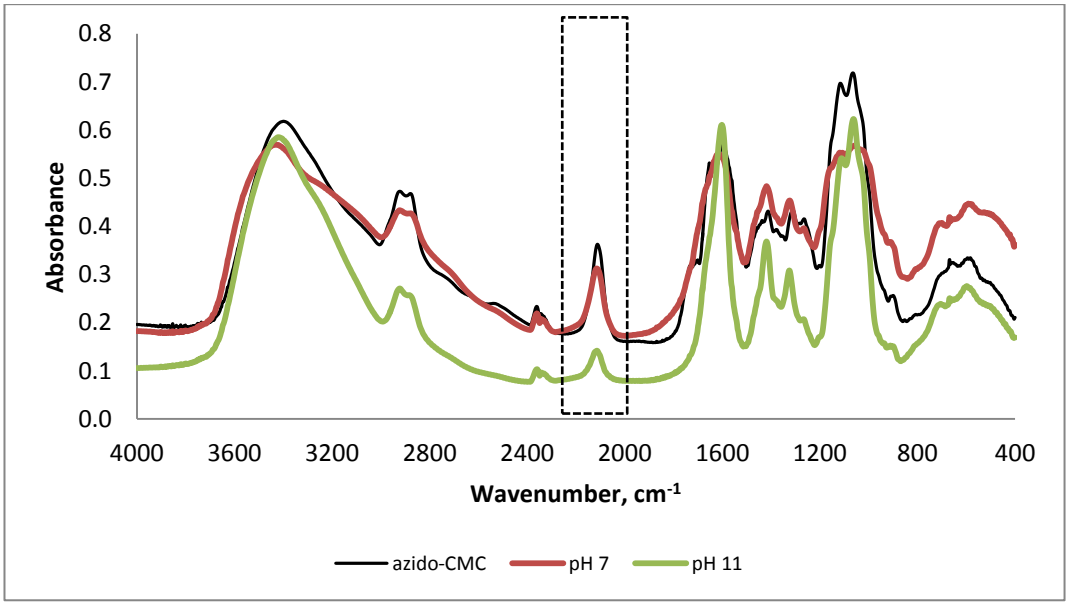


Figure 4-11. FTIR spectra illustrating the chemical stability of azido-CMC after alkaline treatment at 80°C for 15 minutes. The characteristic azide stretching bands are indicated by the framed box.

Mechanically treated fibre samples were analyzed with UV Raman spectroscopy, and obtained spectra did not differ from those collected before the treatment, as

illustrated in Figures 4-12 and 4-13. The “clicked” sample was also tested under UV light and appeared to be intensively fluorescent, as depicted in Figure 4-14.

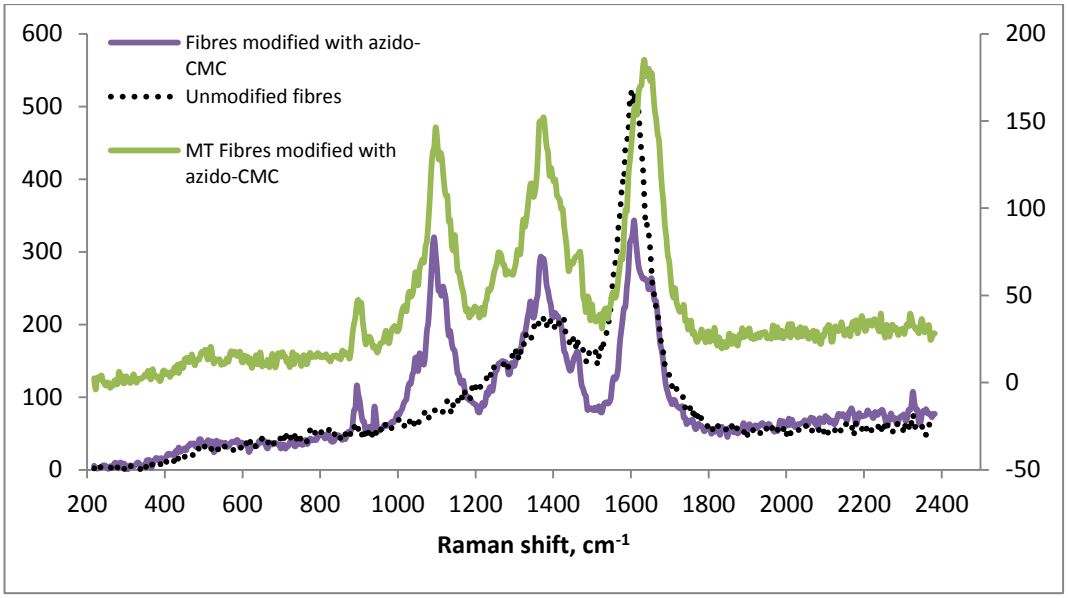


Figure 4-12. UV Raman spectrum of mechanically treated (MT) fibres modified with azido-CMC (green line). Spectra of unmodified fibres (dashed black line) and fibres modified with azido-CMC (blue line) are presented as a reference.

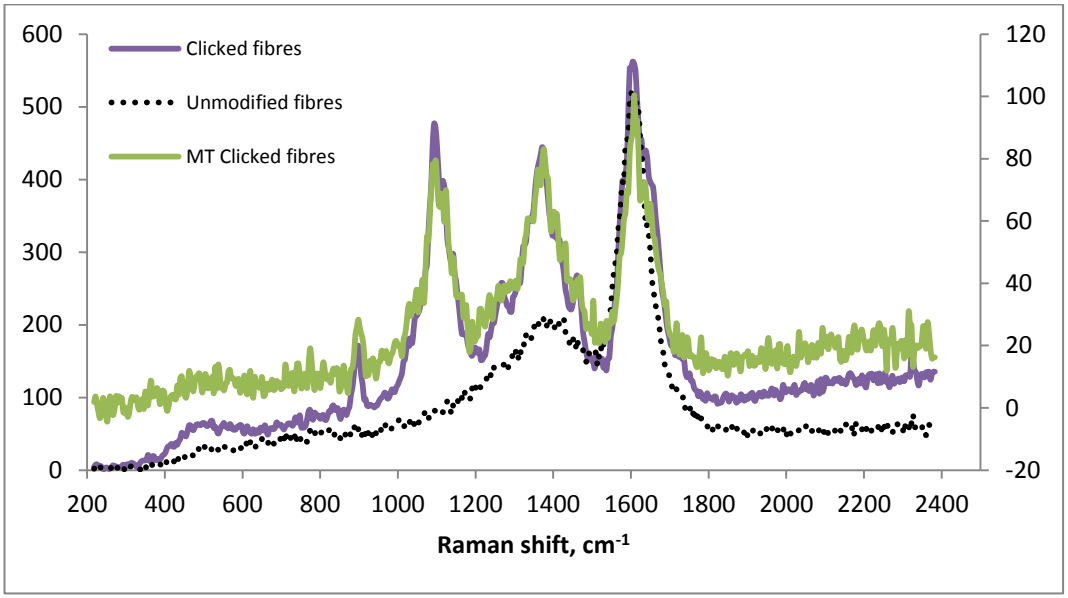


Figure 4-13. UV Raman spectrum of mechanically treated (MT) “clicked” fibres (green line). Spectra of unmodified fibres (dashed black line) and fibres modified with azido-CMC (blue line) are presented as a reference.

In addition, the click reaction was successfully performed with mechanically treated azido-CMC-modified fibre sample (Figure 4-15). It can be concluded that modifications applied are capable of withstanding even harsher mechanical forces than those taking place in papermaking process.

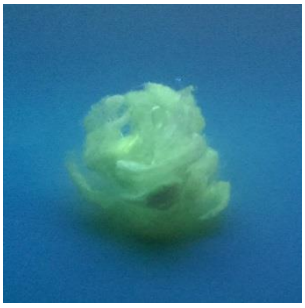


Figure 4-14. Photographic image of fluorescent "clicked" fibres after mechanical treatment

Figure 4-15. Photographic images of mechanically treated azide-modified viscose fibres: control sample(reaction with fluorescent probe in absence of Cu(I) catalyst, left) and "clicked" sample (reaction with fluorescent probe using Cu(I) catalyst, right)

4.5 Fibre properties

The standard set of fibre properties was measured with Lenzing Instruments Vibroscop 400 and Vibrodyn 400 before and after modifications. As received, fibres were marked with titer value of 1.7 dtex; however, the average based on the titer measurements in dry and wet conditions appeared to be slightly lower than that (1.42 dtex). Compared to unmodified fibres, there is a slight increase in titer and force values for fibres modified with CMC-adduct and "clicked" fibres in

both dry and wet conditions. Considering that such measurements imply numerous trials to achieve results of high accuracy, and only 10 trials per sample were carried out in this work, the obtained data is not highly trustworthy and is likely to contain experimental errors.

Table 4-4. Summary of fibre properties

	Dry (conditioned)				**Wet**			
Fibre sample	Titer, dtex	Force, cN	Elong., %	Ten., cN/tex	Titer, dtex	Force, cN	Elong., %	Ten., cN/tex
unmod.	*1.51*	*3.45*	*22.46*	*22.92*	*1.32*	*1.49*	*21.44*	*11.42*
azido-CMC-mod.	1.87	3.48	14.31	18.44	1.75	2.02	20.68	11.55
clicked	1.84	3.95	21.69	21.53	1.95	1.97	22.72	10.18

5 CONCLUSIONS

Within this study, the surface of the viscose fibres was modified by irreversible adsorption of functionalized carboxymethyl cellulose and subsequent covalent attachment of clickable functional groups onto the pre-modified fibres by means of the CuAAC click reaction. The adsorption efficiency of CMC and its derivatives onto viscose in different reaction conditions was assessed by various chemical analysis techniques. The effect of modification was also studied from the chemical and mechanical stability points of view. The findings demonstrated the substantial behavioural difference between the studied regenerated cellulose and widely researched wood pulp and cotton cellulose.

The first step of the viscose modification procedure - the functionalization of CMC - produced clear change in the molecular structure of CMC, resulting in increase of nitrogen content, as demonstrated by the elemental analysis. In addition, FTIR, UV Raman and H-NMR spectroscopic analyses revealed characteristic signals of alkyne, azide and amide bonds. Next step, which was adsorption of CMC and its functionalized derivatives onto viscose fibres, was analyzed with UV Raman, XPS and elemental analysis, where the latter did not reveal any changes in nitrogen content on the fibre surface which may be explained by the relatively low nitrogen content with respect of the fiber matrix (below the detection limit of elemental analysis). It is also worth mentioning that the conductometric titration failed to provide any reliable data (from respective charges) even after multiple trials and, therefore, is not discussed in this work. Nevertheless, Raman spectroscopy and XPS results indicated changes on the surface of the viscose fibres. Firstly, the Raman spectra of the fibres modified with carboxymethyl cellulose and its azide- and alkyne-derivatives illustrated the appearance of new signals which were not detected for the reference sample of

unmodified fibres. Although the XPS analysis appeared challenging to perform due to the viscose charge, it did detect traces of nitrogen at least in the most nitrogen-rich “clicked” fibre sample. Additional quantitative assessment of adsorption efficiency resulted in average figures slightly lower than those obtained in earlier studies on modification of cellulose pulp; however, the effect of varying reaction conditions onto adsorption appeared unclear. In contrast to temperature and electrolyte concentration dependency that was observed in the cellulose pulp, modification of regenerated cellulose did not demonstrate any distinguishable behavioural pattern. One potential explanation for such uncertainties could be the difference in crystal structures of cellulose I and cellulose II. Chemical stability of amide bonds tested in mild to medium alkaline conditions resulted in positive outcomes, eliminating the concern of amide bond hydrolysis. Moreover, the mechanical treatment indicated no reducing effect on the click chemistry investigated. To conclude, the modification of viscose fibres by means of combining adsorption of carboxymethyl cellulose with click chemistry showed promising results, and can be seen as a novel method with high potential.

5.1 Further research

The adsorption efficiency of CMC and its "clickable" derivatives onto viscose fibres in different reaction conditions together with the chemical and mechanical stabilities of the modification were evaluated within this study; however, the behaviour of regenerated cellulose needs deeper investigation for better understanding. Moreover, easier and more reliable methods need to be established for the evaluation of the modification process. Some additional related topics that could interest the manufacturer are recommended for the further research: 1) effect of the concentration of the Ca^{2+} cations for multi-layered modifications, 2) CMC adsorption on cationic fibres in absence of electrolyte solution that could be studied on the manufacturer's unique ion exchange fibres, and 3) application of other click reactions, such as thiol-ene and Cu-free SPAAC that eliminate application of the copper catalyst.

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APPENDIX A

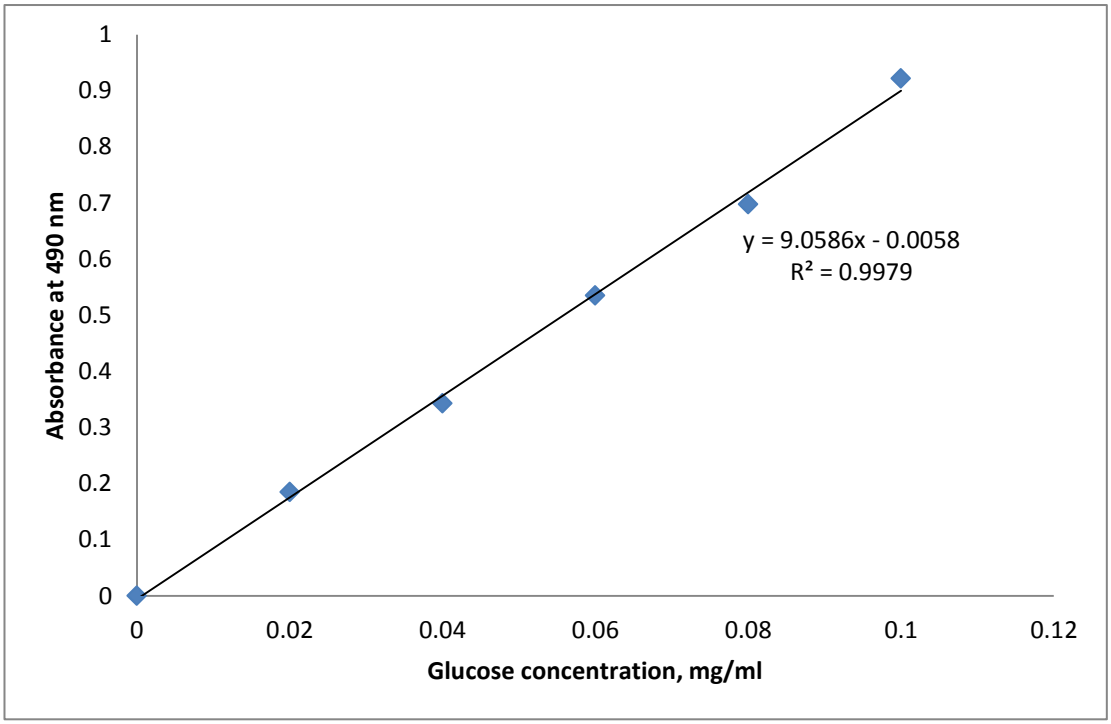

Figure A-1. Standard curve for total carbohydrates

Table A-1. Light absorbance of glucose solutions at wavelength of 490 nm

Sample	ABS at 490 nm	mg gluc./ml
0	0	0
1	0.185	0.02
2	0.343	0.04
3	0.535	0.06
4	0.698	0.08
5	0.922	0.1

Amount of residual CMC in a test tube, mg/ml, was calculated as:

$$\frac{ABS - b}{a}$$

Where *ABS* is absorbance at 490 nm, *a* and *b* are the coefficients of the line equation *y=ax+b* equal to 9.0586 and - 0.0058, respectively.

Amount of residual CMC in 50 ml of initial filtrate (f_1), mg/50 ml, as adsorption was carried out in 50 ml of electrolyte solution:

$$f_1 = \frac{ABS - b}{a} \cdot DF \cdot 50$$

Where *DF* is the dilution factor equal to 1/0.2=5 (0.2 ml of initial filtrate diluted to the volume of 1 ml). Every sample was additionally washed and filtered twice, thus CMC residues in subsequent filtrates f_2 and f_3 were calculated analogically.

Percentage of adsorbed CMC was calculated from the difference between initial amount adsorbed, 20 mg, and the sum of the residues in filtrates:

$$\frac{20 - \sum f_n}{20} \cdot 100\%$$

Nitrogen content in modified CMCs was found as follows:

$$N = \frac{n \cdot Mw_N}{(Mw_{AGU} + Mw_{conj.am.}) \cdot 0.7}$$

Where *n* is the amount of nitrogen atoms in the moiety-bearing amine compound and 0.7 is the initial DS of unmodified CMC. Thus, for azido-CMC:

$$N_{az} = \frac{4 \cdot 14}{(162 + 259.28) \cdot 0.7} = 0.19$$

And for alkyne-CMC:

$$N_{alk} = \frac{1 \cdot 14}{(162 + 96.11) \cdot 0.7} = 0.086$$

On the example of the highest absorption efficiency estimated (Table 4-3) nitrogen content per approximately 1 g of fibres modified with azido-CMC can be calculated as:

$$\frac{m_{CMC} \cdot \%adsorbed}{m_{fibres}} \cdot N_{az} \cdot E$$

Where *E* is the CMC functionalization efficiency equal to 60%. Thus,

$$\frac{20mg \cdot 34.4\%}{1000mg} \cdot 0.19 \cdot 0.6 = 0.08\%$$

APPENDIX B

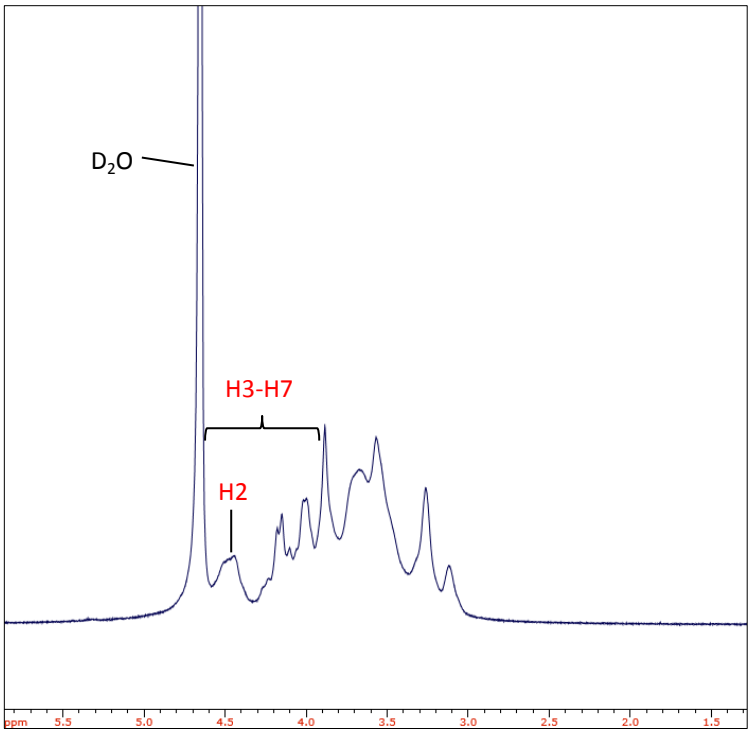

Figure B-1. H-NMR spectrum of unmodified CMC

Table C-1. XPS results of the analyzed viscose fibres

	Wide atomic concentrations (AC, in atomic %)						HiRes carbons				
Vamas Dec13-Jan14	**#**	**C 1s**	**O 1s**	**Si 2p**	**N 1s**	**notes**		**C-C**	**C-O**	**C=O**	**COO**	**<CC>**
viscose control	**7**	65.4 %	34.0 %	0.6 %			7	16.9 %	45.1 %	26.5 %	***11.5 %***	
	16	61.9 %	38.1 %				16	17.6 %	50.8 %	22.5 %	***9.2 %***	
viscose + CMC	3	60.7 %	39.3 %				3	11.8 %	63.6 %	21.4 %	3.2 %	
	6	59.9 %	40.1 %				6	8.9 %	61.8 %	22.9 %	***6.4 %***	
viscose, clicked	2	64.2 %	35.8 %		trace	Detection limit is too	~~2~~	20.1 %	56.2 %	19.7 %	4.0 %	
	9	62.7 %	36.5 %	0.9 %	trace	high, close to 1 at%	9	23.8 %	54.5 %	18.9 %	2.9 %	
	10	63.0 %	35.6 %	0.7 %	0.7 %		10	21.5 %	52.6 %	22.0 %	3.9 %	
viscose + azido-CMC	**5**	61.4 %	38.6 %				5	11.8 %	55.6 %	24.3 %	***8.3 %***	
	18	62.2 %	37.9 %				18	12.6 %	60.4 %	21.1 %	5.9 %	
viscose + alkyne-CMC	**8**	61.1 %	38.9 %				8	9.0 %	58.0 %	26.3 %	***6.7 %***	
	9	68.0 %	32.0 %		trace	contaminated spot?	~~9~~	***32.1 %***	49.8 %	15.1 %	3.0 %	
	17	60.2 %	39.8 %				17	13.6 %	62.5 %	20.5 %	3.4 %	
Aalto XPS ref		**C 1s**	**O 1s**					**C-C**	**C-O**	**C=O**	**COO**	**<CC>**
Whatman	1	59.2 %	40.9 %				1	4.0 %	75.6 %	18.3 %	2.1 %	
First set (2013)	11	59.5 %	40.5 %				11	4.7 %	75.2 %	18.1 %	2.1 %	**4.4 %**
Whatman	**1**	57.7 %	42.3 %				1	4.0 %	72.2 %	21.3 %	2.5 %	
Second set (2014)	**11**	59.1 %	40.9 %				11	5.1 %	70.8 %	20.2 %	3.9 %	**4.7 %**
	12	55.3 %	44.7 %				12	4.4 %	75.4 %	18.3 %	1.9 %	
	19	58.9 %	41.1 %				19	5.2 %	74.7 %	18.1 %	2.0 %	